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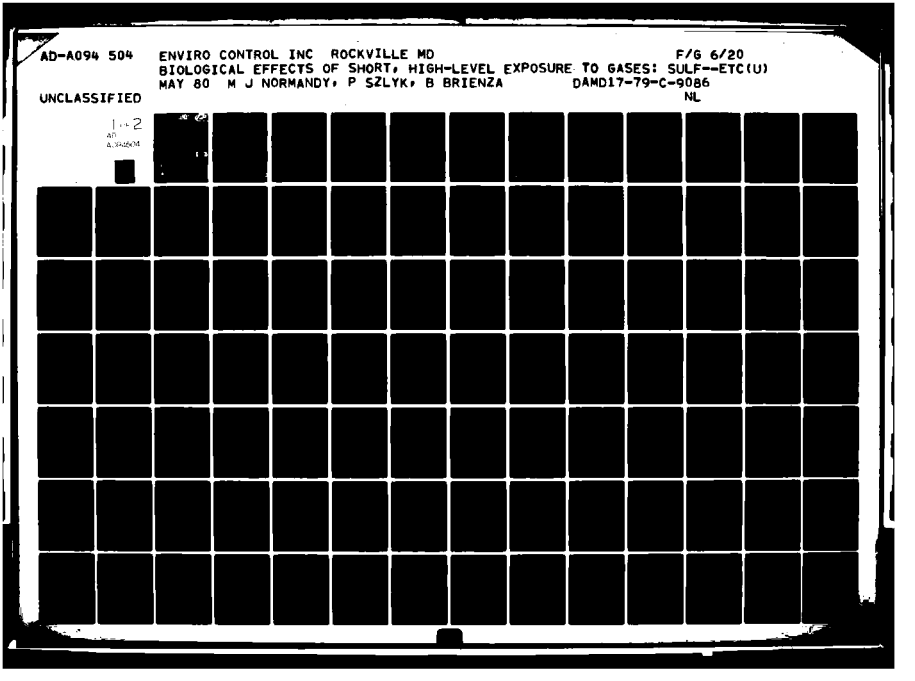

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U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

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BIOLOGICAL EFFECTS OF SHORT, HIGH-LEVEL EXPOSURE TO GASES: SULFUR DIOXIDE

PHASE REPORT

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This project was one of four under the same contract; the others covered were ammonia, carbon monoxide, and the nitrogen oxides.

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Sulfur Dioxide	Acute	Effects	Biological
Exposure	Chronic	Behavioral	
Concentration	Intermittent	Toxicity	
Dose	Continuous	Physiology	

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

This report presents an analysis and synthesis of the available literature concerned with possible health effects of exposures to sulfur dioxide. The U.S. Army is concerned with short, high-level exposures to sulfur dioxide that may exceed present threshold limit values of the American Conference of Governmental Industrial Hygienists (5 ppm, 13 mg/m^3) as a time-weighted average.

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The organ systems primarily affected by exposure to sulfur dioxide are the respiratory tract and the eyes. Certain neurologic effects (including suppression of dark adaptation and decreased light sensitivity) are of unknown significance and warrant further study. Below about 5 ppm, there are no significant irritant or pulmonary effects. Between 5 and 8 ppm (13 and 20.8 mg/m^3), most people will experience coughing, moderate irritation of the eyes, nose, and throat, and bronchoconstriction. At about 10 ppm (26 mg/m^3), moderate to severe eye irritation, copious lacrimation, and nasal and chest irritation will occur. At 20 to 30 ppm (52 to 78 mg/m^3), intense lacrimation, respiratory tract irritation, bronchoconstriction, epistaxis, sneezing, coughing, and hemoptysis may occur.

With nasal breathing, about 99% of inspired sulfur dioxide is absorbed by the nasal mucosa and only 1% penetrates the lower airways. With mouth breathing, the effects of sulfur dioxide at a given concentration may be intensified. One reason is that absorption by oral mucosa, although variable, is lower than by nasal mucosa, resulting in increased penetration by sulfur dioxide into the lower airways. Another reason is that during excitement and exercise, when mouth breathing usually occurs, respiration increases so that a greater total amount of sulfur dioxide will be inspired. Thus, sulfur dioxide threshold concentrations for specific effects will decrease and the severity of effects at given sulfur dioxide concentrations will increase.

Personnel may become rapidly tolerant to the irritant effects within 3 to 5 minutes and to the pulmonary mechanical effects of the gas within 10 to 15 minutes, at concentrations up to 25 to 30 ppm (65 to 78 mg/m^3). This adaptation will be negated by further abrupt concentration increases; a reestablished tolerance at higher unspecified sulfur dioxide levels has been speculated. Gradual increases in concentration may reduce or completely eliminate both the irritant and mechanical pulmonary effects. Repeated, intermittent exposure will reduce tissue pathology expected with continuous or prolonged exposure, presumably due to tissue repair during the periods of nonexposure.

The most significant irritant effect, from the military standpoint, may be moderate lacrimation that will occur at about 5 ppm (13 mg/m^3) and intense lacrimation at about 10 ppm (26 mg/m^3). This effect may impair operational efficiency in any tasks involving visual discrimination.

Extreme variability exists in concentrations reported to be tolerable, probably because of individual sensory variation and motivation.

The potential for delayed irreversible effects due to sulfur dioxide exposure has been suggested in studies of chronic industrial exposure and human and animal overexposures.

With daily intermittent exposures to sulfur dioxide at concentrations below 25 ppm (65 mg/m^3), expected effects will be immediate, reversible irritant effects and significant only to the degree that they impair operational efficiency.

EXECUTIVE SUMMARY

The purpose of this project is to characterize the biologic responses to short, high-level exposures to four gases (ammonia, carbon monoxide, sulfur dioxide, and the nitrogen oxides) that may be associated with certain Army weapons systems and troop field training activities. This report analyzes and synthesizes the available literature on possible health and performance effects of exposure to sulfur dioxide.

Exposure of soldiers to sulfur dioxide may occur during training with the various weapons systems, under certain field training activities, or during combat. Armored vehicle crewmen may be especially vulnerable to these exposures because of the close confinement and sometimes poor ventilation inside the vehicles and because of the proximity of personnel to the emission sources. Exposures are expected to be intense (above the threshold limit value of 5 ppm (13 mg/m^3) recommended by the American Conference of Governmental Industrial Hygienists), brief (1 hour or less), and repeated (one to six times daily for periods of 1 to 14 days).

Threshold limit values for use in the workplace have limited application in the military setting; the basis for their selection is the protection of chronically exposed workers against nose and throat irritation and the minimization of discomfort in uninured individuals. Selection of maximum allowable concentrations of sulfur dioxide for use in the military should more appropriately be based on prevention of casualties (i.e., immediate incapacitation or delayed health effects) and any effects that would impair operational efficiency.

In 1966, the National Research Council recommended emergency exposure limits for sulfur dioxide (NRC Committee on Toxicology, unpublished). No irreversible damage is expected to occur to the individual at the stated exposure limits if the incidence of exposure is rare (once or twice in a lifetime). The recommended emergency exposure limits were:

- 30 ppm (78 mg/m^3) for 10 minutes
- 20 ppm (52 mg/m^3) for 30 minutes
- 10 ppm (26 mg/m^3) for 60 minutes
- 5 ppm (13 mg/m^3) for 24 hours.

This report attempts to identify threshold levels at which various effects may be expected to occur; the nature and extent of possible effects when such levels are exceeded; and gaps and inconsistencies in available data so that areas in which follow-on research may be required can be determined.

The data on which this report is based were derived by the collection, critical review, and evaluation of published literature and research reports. The main sources of information were the various computer data bases, especially MEDLINE and its back files, TOXLINE,

TOXBACK, NTIS, and NIOSHTIC. The data of greatest interest are contained in studies of short, high-level exposures in humans. These reports deal mainly with the immediate, reversible, irritant, and respiratory effects of exposure to sulfur dioxide. Data on possible effects of prolonged exposure, either in continuous or repeated doses, are contained in both human and animal studies. Reports of accidental human exposure have also been reviewed. While the exposure levels in such instances were probably well above any likely to be encountered in the military environment, the reports are instructive with respect to the nature and extent of the pathology resulting from inhalation of sulfur dioxide at very high concentrations.

The organ systems primarily affected by exposure to sulfur dioxide are the respiratory tract and the eyes. The irritant effects are immediate, beginning with the onset of exposure, are primarily concentration-dependent, and, except possibly under conditions of prolonged exposure, are probably completely reversible at concentrations of 25 ppm (65 mg/m^3) and below for exposure periods of 1 hour or less.

Below 5 ppm (13 mg/m^3), there does not appear to be significant adverse effects. At 1 to 3 ppm (2.6 to 7.8 mg/m^3), sulfur dioxide will be detectable, first by taste, then by odor. Although suppression of dark adaptation and decreased light sensitivity have been reported after exposures to 0.2 to 1.1 ppm (0.52 to 2.85 mg/m^3), the biologic significance of these reported changes has not been established. The severity and significance of these effects have not been established. Between concentrations of 5 and 8 ppm (13 to 20.8 mg/m^3), most individuals will experience coughing, a moderate degree of irritation to the eyes, nose, and throat, and bronchoconstriction. However, these effects may not be so severe as to interfere with mission accomplishment. At about 10 ppm (26 mg/m^3), moderate to severe eye irritation, copious lacrimation, and nasal and throat irritation will occur. At 20 to 30 ppm (52 to 78 mg/m^3), intense lacrimation, respiratory tract irritation, bronchoconstriction, epistaxis, sneezing, cough, chest constriction and hemoptysis usually occur. Individuals rarely tolerate these effects for more than 15 minutes.

The threshold concentrations for these effects vary primarily with the mode of inhalation (mouth or nose) and minute volume. Mouth breathing and increased respiratory rates and tidal volumes will decrease the threshold concentrations of sulfur dioxide necessary to elicit certain toxic effects and will increase the severity of those effects at given concentrations.

Personnel may become rapidly tolerant to sulfur dioxide at concentrations up to 25 ppm (65 mg/m^3). Responses to its irritant and mechanical pulmonary effects (e.g., bronchoconstriction) diminish within 3 to 5 and 10 to 15 minutes, respectively. Gradual acclimation to greater concentrations occurs only after several weeks or months of repeated, daily exposure. Further exposure to abruptly increased gas concentrations negates this acclimation, and adaptation will have to be reestablished at the higher concentration. At concentrations above 25 to 30 ppm (65 to 78 mg/m^3), the rapid adaptive response has not been observed.

Repeated, intermittent exposure (as opposed to continuous, prolonged exposure) may produce a lower incidence of tissue pathology induced by sulfur dioxide exposure, presumably because of repair of pulmonary tract lesions during the periods of nonexposure. Thus, the greater the interval between exposures, the less severe the damage to the respiratory tract and other tissue should be.

Perhaps the most significant irritant effect from the military standpoint is the moderate lacrimatory effect that will occur at about 5 ppm and the intense lacrimation that will occur at about 10 ppm. Lacrimation may be expected to impair operational efficiency because of interference with tasks involving visual discrimination (including reading instruments and maps and gun sighting).

Neurologic effects may occur at sulfur dioxide concentrations below the level of sensory perception. At less than 1 ppm, some suppression of dark adaptation, decreased light sensitivity, disruption of alpha brain waves, and elevation of optical chronaxy have been reported.

There is extreme variance in the concentrations reported to be tolerable to uninured subjects. An early study reported concentrations of 500 to 514 ppm (1300 to 1336 mg/m^3) were tolerated for 2 hours. In another early study, 300 ppm (780 mg/m^3) was considered intolerable for any length of time. The consensus of numerous recent studies is that 20 to 30 ppm (52 to 78 mg/m^3) is the maximum tolerable concentration for 15 minutes or more.

Sulfur dioxide is rapidly absorbed by mucosal tissue in the mouth and nose, with the greater absorptive capacity demonstrated in the nasopharyngeal mucosa. Up to 99 percent absorption of the inspired gas has been observed in various human and animal studies in which sulfur dioxide was inhaled by nose. With nasal inhalation, the threshold level of sulfur dioxide that produces coughing indicates that the minor amounts that have penetrated the nasal airways are sufficient to activate laryngeo- tracheal receptors. Data do not suggest that the bronchi, bronchioles, or alveoli are affected.

In situations of increased physical exertion where mouth breathing is more likely to prevail, less sulfur dioxide will be absorbed by mucosal tissue than during nasal inhalation. Thus, a greater proportion of the inspired gas will be available to penetrate the lower pulmonary passages and elicit toxic effects.

Studies of the interactions of sulfur dioxide with various aerosols have produced conflicting results. Data suggest, however, that potentiation of the adverse effects of sulfur dioxide exposure becomes more likely with the decreasing particle size of the aerosol.

The major evidence of possible irreversible effects is found in studies of chronic industrial exposure and accidental overexposures in humans and monkeys. The intensity of these delayed irreversible effects is primarily determined by the gas concentration.

Definitive answers to questions about possible reversible or irreversible effects in humans due to repeated, short exposures to high levels of sulfur dioxide will require additional research. While the animal data create some concern about the possibility of tracheal mucosal damage under conditions of repeated, worst-case exposures, it has not been concluded that such effects are probable. Rather, effects are expected to be immediate, reversible, in the category of harassing, and significant only to the extent that they may affect operational efficiency to some degree. The possibility of delayed systemic damage by metabolites of sulfur dioxide, as noted in several animal studies, also warrants further study.

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ABBREVIATIONS

Abbreviation	Meaning	Unit of Expression
C	Compliance	liter/cm H_2O
Ct	Product of concentration and time	ppm x minutes
CtD_{50}	Product of concentration and time resulting in the delayed death of 50% of a group of test animals	ppm x minutes
$FEV_{1.0}$	Forced expiratory volume in one second	liters
FRC	Functional residual capacity	liters
Gaw	Airways conductance	liters/second/cm H_2O
HR	Heart rate	beats/minute
LC	Lethal concentration	ppm
LCt_{50}	Product of concentration and time resulting in the death of 50% of a group of test animals	ppm x minutes
Lt_{50}	Time within which death will occur in 50% of a group of test animals exposed to a given concentration of a test substance	minutes, hours, days
MAC	Maximum allowable concentration	ppm
MEFR	Maximum expiratory flow rate	liters/second
$MEF_{50\%\ VC}$	Maximum expiratory flow rate at one-half vital capacity	liters/second
MMEF	Maximum midexpiratory flow rate	liters/second
MCtD	Mean product of concentration and time when exposure is extended until the death of all test animals	ppm x minutes
MTD	Median time of death	minutes, hours, days

Abbreviation	Meaning	Unit of Expression
PFR	Pulmonary flow resistance	cm H_2O/liter/ second
P_{aO_2}	Ideal partial pressure of oxygen in the artery	cm H_2O
P_{aCO_2}	Ideal partial pressure of carbon dioxide in the artery	cm H_2O
ppm	Parts per million	any number
R_L	Total pulmonary resistance	cm H_2O/liter/ second
R_{LX}	Resistance of the larynx	cm H_2O/liter/ second
R_N	Resistance of the nose	cm H_2O/liter/ second
RR	Respiratory rate	breaths/minutes
Rrs-e	Total respiratory system flow resistance during expiration	cm H_2O/liter/ second
Rrs-i	Total respiratory system flow resistance during inspiration	cm H_2O/liter/ second
t_e	Expiratory time	seconds
t_i	Inspiratory time	seconds
TLV	Threshold limit value	ppm
TWA	Time weighted average	ppm/hour
VC	Vital capacity	liters
V_t	Tidal volume	liters
V-l min	Minute volume	liters
W-e	Mechanical work of breathing during expiration	dynes/cm^2 x cm^3
W-i	Mechanical work of breathing during inspiration	dynes/cm^2 x cm^3

I. INTRODUCTION AND BACKGROUND

A. U.S. ARMY EXPOSURE SETTING FOCUS

The overall problem addressed by this project is the potential exposure of soldiers to toxic gases, including carbon monoxide (from engine exhaust, auxiliary generators, and explosives), ammonia (from explosives, especially those formulations containing nitroguanidine), oxides of nitrogen (from explosives), and oxides of sulfur (from explosives and engine exhaust). The exposures are likely to be intense (above present threshold limit values (TLV) of the American Conference of Industrial Hygienists), brief (1 hour or less), and repeated (one to six times daily for periods of 1 to 14 days). Such exposures may occur in the crew compartments of weapons systems such as tanks during the training of soldiers with the various weapons systems, during certain field training activities, or during actual combat.

This report presents a synthesis and analysis of the available literature concerned with possible health and performance effects of exposure to sulfur dioxide (SO_2), including lethality, possible immediate and reversible irritant effects, and/or delayed and irreversible effects, and neurologic effects. An effort has been made to identify threshold levels at which effects may be expected to occur, applicable concentration and concentration-time (Ct) relationships, the nature and extent of effects when such levels are exceeded, and gaps and inconsistencies in available data to determine the areas in which follow-on research may be required.

B. EXISTING OCCUPATIONAL AND PUBLIC HEALTH STANDARDS

In 1966, the National Research Council recommended emergency exposure limits for sulfur dioxide (NRC Committee on Toxicology, unpublished). No irreversible damage is expected to occur to the individual at the stated exposure limits if the incidence of exposure is rare (once or twice in a lifetime). The recommended emergency exposure limits were:

- 30 ppm (78 mg/m^3) for 10 minutes
- 20 ppm (52 mg/m^3) for 30 minutes
- 10 ppm (26 mg/m^3) for 60 minutes
- 5 ppm (13 mg/m^3) for 24 hours.

The present federal occupational standard established by the Occupational Safety and Health Administration (OSHA) for SO_2 (5 ppm* as a

*Throughout the remainder of the report, SO_2 concentrations are expressed as parts per million (ppm). To convert ppm to mg/m^3, multiply ppm by 2.6. This constant is derived by dividing the molecular weight of sulfur dioxide (64 g) by the volume that one mole of gas would occupy at 25°C (298°K) and one atmosphere of pressure, multiplied by $1/m^3$ (to convert m^3 units).

time weighted average, TWA) was adopted from the TLV of 5 ppm recommended by the American Conference of Governmental Industrial Hygienists (ACGIH) in 1973.[1] The selection of the TLV was based on documentation that 5 ppm would not produce respiratory tract irritation in most workers and would cause only minimal effects in workers who are sensitive to SO_2. However, in 1974 the National Institute of Occupational Safety and Health (NIOSH) recommended a standard of 2 ppm as a TWA on the basis of documentation in both animals and humans of significant changes in respiratory mechanics including reductions in various measures of pulmonary function at concentrations of SO_2 over 2.5-3 ppm.[2] In the 1979 notice of intended changes in 1980, ACGIH proposed that the current TLV be lowered to 2 ppm and that a short-term (15 minute) exposure limit (STEL) of 5 ppm be adopted.

The TLVs developed for use in the workplace would appear to have limited application in the various military exposure scenarios under consideration here. Such TLVs are established to prevent adverse health effects in workers exposed 8 hours per day, 5 days a week, over a working lifetime. In contrast, soldiers (whether in training or combat) are likely to receive intense but brief exposures, repeatedly over a period of several days, followed by periods of no exposure lasting days, weeks, or months. In establishing a maximum allowable concentration (MAC) for sulfur dioxide for military application, the main problem is to determine levels that will result in casualty production (i.e., either immediate incapacitation or delayed health effects) or that will impair immediate operational efficiency. In the case of SO_2, concentrations and durations of SO_2 exposure that will reduce the capacity of the soldier to perform physically demanding tasks because of compromised pulmonary function (even though temporary) must be considered.

C. CHEMICAL PROPERTIES OF SULFUR DIOXIDE

Sulfur dioxide is an acid anhydride which reacts with water to form sulfurous acid (H_2SO_3):

$$SO_2 + H_2O \rightarrow H_2SO_3$$

At 35°C (approximate human body temperature) the solubility of this gas is 6.4 g per 100 g of water. With further oxidation, sulfurous acid forms the stronger acid, sulfuric acid (H_2SO_4):

$$2\ H_2SO_3 + O_2 \rightarrow 2\ H_2SO_4$$

The interaction of either sulfurous or sulfuric acid with the moist areas of an animal's respiratory tract or corneal epithelium can result in the irritation of mucosal tissue.

D. MEASUREMENT OF SULFUR DIOXIDE CONCENTRATIONS

Various methods are available for determining sulfur dioxide concentrations in air.[3] The West-Gaeke method and the conductometric methods are currently the most commonly employed in the studies discussed in this report. These and other basic methods are presented.

1. West-Gaeke Method

The West-Gaeke method, introduced in 1956, is a colorimetric method specific for sulfur dioxide and sulfite salts. Sulfur dioxide is absorbed in dilute aqueous sodium tetrachloromercurate to form the nonvolatile dichlorosulfito-mercurate ion, which then reacts with formaldehyde and bleached pararosaniline to form red-purple pararosaniline methylsulfonic acid. The color intensity of this compound, which is proportional to the concentration of sulfur dioxide, can be measured at a wavelength of 560 nm. This method can be best used to determine sulfur dioxide in the air at a concentration range of 0.002 ppm to 5 ppm.

Two improvements of the West-Gaeke method have been developed to determine sulfur dioxide in ambient air. These give greater sensitivity and reproducibility, as well as adherence to Beer's Law (the proportionality of a solution's concentration and optical density at a given wavelength) throughout a greater working range, than does the original West-Gaeke method. One improvement is the purification and standardization of the pararosaniline dye to reduce variability. The other is the addition of phosphoric acid in the final color development to control pH. Interferences from nitrogen oxides, ozone, and heavy metals are minimal, and laboratory results are reproducible to within 4.9 percent (at 95 percent confidence level). This has been adopted as EPA's standard method for ambient air analysis.

2. Conductometric Methods

Conductometric methods of sulfur dioxide analysis have been in wide use since the 1960s. The basis of these methods is the oxidation of sulfur dioxide to sulfuric acid by aqueous hydrogen peroxide, and the subsequent measurement of the increased electrical conductivity of the solution. This is a general technique; precautions must be taken to eliminate other pollutants that can affect the conductivity of the solution. For example, the presence of acidic gases such as the hydrogen halides will increase the conductivity of the solution and give erroneously high SO_2 values. Weakly acidic gases such as hydrogen sulfide cause practically no interference because of slight solubility and poor conductivity, and nitrogen dioxide produces minimum interference because it is poorly absorbed. Sulfuric acid mist is not efficiently collected in the usual gas scrubber and therefore is not measured. Ammonia interferes with electroconductivity measurements by neutralizing the acid and forming ammonium sulfate:

$$2\ NH_3 + H_2SO_4 \longrightarrow (NH_4)_2SO_4$$

Conductivities that are then recorded are inaccurately low.

Conductometric methods can be automated to obtain instantaneous readings. Air is drawn through either acidic hydrogen peroxide solution or deionized water, and the sulfur dioxide concentration is estimated from the conductivity of the final solution.

3. Acid Titration Methods

In this method, the air sample is bubbled though hydrogen peroxide solution. Any sulfur dioxide present forms sulfuric acid, which is then titrated with standard alkali. The presence of other acidic gases in the sample will lead to erroneously high results, and alkaline gases or reactive basic solids give erroneously low results. This technique may be automated.

4. Early Methods

The earliest method for determining sulfur dioxide was based on its ability to reduce a starch-iodine solution. This method was considered to be reasonably accurate in the range of 0.8 ppm to 3 ppm, and it was later modified to cover the range of 0.1 ppm to 60 ppm. This method has not been automated.

Sulfur dioxide can also be determined by a fuchsin-formaldehyde method in which the sample is collected in sodium hydroxide solution in glycerol-water. Another procedure made use of collection of the gas in a potassium chlorate solution, and analysis was probably by nephelometric analysis of barium sulfate or turbidimetric analysis of lead sulfate. In still another method, sulfur dioxide is absorbed in a solution of barium chloride, and light transmission through the turbid solution, which is inversely related to the sulfur dioxide concentration, is recorded.

Another method for determining ozone and sulfur dioxide involves liberation of iodine by ozone from an iodine solution in one channel of an analyzer and the consumption of iodine by sulfur dioxide in a second analyzer channel.

5. Comparison of Methods

In the United States, the colorimetric West-Gaeke and automated conductometric methods are the most frequently used methods for SO_2 measurement.

The values obtained by the various methods of measuring sulfur dioxide may not always correlate well with one another, because they do not measure the same thing and because interfering substances are present in varying amounts from time to time. It is generally assumed that sulfur dioxide is the major constituent in air that will respond to any of the methods discussed; some recent studies show that the assumption may not be entirely valid.

Recently, the conductivity and the West-Gaeke colorimetric pararosaniline techniques have been evaluated in depth. No consistent relationship between the two measurements has been found, although the conductometric method usually gave higher values. This is expected, since the West-Gaeke method is considered to be more specific and less susceptible to interference from outside contaminating substances.

II. APPROACH TO THE PROBLEM

A. OVERVIEW

The approach to the work involved the following major tasks:

- Identification of information sources
- Preliminary screening of information before acquisition
- Assessment of the availability of sufficient literature to perform remaining work elements
- Acquisition of the literature
- Critical review of documents for scientific validity
- Evaluation of biologic response data in terms of behavioral, performance, and health effects, including immediate and delayed, and reversible and irreversible effects
- Synthesis of biologic effects, recommendation of occupational health effects criterion, and identification of data gaps/research needs.

The main sources of information were the various computer data bases, especially MEDLINE and its backfiles, TOXLINE, TOXBACK, NTIS, and NIOSHTIC. The computer search was performed by first selecting key terms describing substance and exposure. Only articles containing one or more terms from each of three sets of key words were retrieved.

All materials resulting from the search of the data bases were screened to identify articles apparently relevant to the study. The screening was based on the content of article abstracts (when available) and on the presence of keywords in article titles. Full-text copies of all apparently relevant articles were then obtained for critical review and evaluation. Information and materials were also obtained from individuals at various institutions and agencies including the University of Buffalo, Harvard Medical School, Environmental Protection Agency, the National Research Council, and NIOSH.

The search of the data bases and the screening of the resulting materials revealed that several studies in the literature related to sulfur dioxide exposure in humans and animals were concerned with short, high-level exposures. These articles were divided into types of exposure (animal or human, laboratory or environmental) and general areas of effects. Key documents were reviewed in detail and other documents were used to corroborate information in the report. A major deficiency was recent data on the lethality of SO_2. Most of these studies were

conducted years ago, and apparently they have been accepted without critical analysis of methodology or results in the light of current technology.

B. LITERATURE ASSESSMENT CRITERIA

The studies included in the Literature Review and Analysis section were evaluated using various criteria, including:

- Adequacy of experimental design
- Appropriateness of procedures
- Statistical validity
- Appropriateness of authors' conclusions about the toxicologic/biologic effects of exposure.

The results of these studies were then included, as appropriate, in the Discussion section of this report.

C. ORGANIZATION OF REPORT

The report is divided into the following sections:

- "Summary of Effects and Conclusions," which presents the main findings of the literature review in summary fashion and identifies significant data gaps and inconsistencies
- "Discussion" of the data presented in the literature, leading to the main findings, gaps, and inconsistencies
- A section of "Suggested Follow-on Work," in which possible additional research is proposed to fill in major gaps in the information or to resolve discrepancies
- "Literature Review and Analysis" (attached as Appendix A), which is a presentation of the purpose, methods, and findings of each key article, followed by a critical analysis, as appropriate, of the experimental design, statistical methods, and appropriateness of the conclusions.

III. SUMMARY OF EFFECTS AND CONCLUSIONS

In presenting the summary of effects data derived from the various studies reviewed, it may be appropriate to again mention the frequency of possible sulfur dioxide exposures to which soldiers may be subjected. The Army is concerned with exposures exceeding present threshold limit values of the American Conference of Governmental Industrial Hygienists for durations of 1 hour or less, one to six times daily, for periods of 1 to 14 days. Given the confined and sometimes poorly ventilated space of various armored vehicles, the assumption is made that concentrations approaching or exceeding the TLV of 5 ppm may be present.

The purposes of this report are to ascertain the nature and extent of health and performance effects at various sulfur dioxide levels, and to identify gaps in the information presently available, which will suggest areas where additional research is required. For the purposes of this report, low levels of sulfur dioxide are defined as concentrations below 5 ppm, moderate levels as 5 to 25 ppm, and high levels as above 25 ppm.

Reported sulfur dioxide concentrations in human studies have ranged from 0.1 to 1500 ppm,[4,5] and in animal studies from 0.1 to 3000 ppm.[4,6] Reports of accidental human[7-11] and animal[12] exposures to extremely high (usually unmeasured) concentrations of sulfur dioxide have provided primarily clinical descriptions and postmortem findings in exposure victims. Several animal studies have used concentrations in the lethal range (e.g., 400 to 3000 ppm, depending on the species).[6,13,14]

A. IMMEDIATE, REVERSIBLE, IRRITANT, PULMONARY, AND NEUROLOGIC EFFECTS

1. Threshold Levels of Responses

Levels at which the various irritant effects of sulfur dioxide have been demonstrated in humans are summarized in Table 1. At these levels, the organ systems primarily affected are the eyes and the upper respiratory tract. The irritant effects are immediate with the onset of exposure, are primarily concentration-dependent, and, except possibly under conditions of prolonged exposure, are probably completely reversible at concentrations of 25 ppm and below if the exposure is brief (1 hour or less).[15]

Neurologic effects may occur even at concentrations below 0.6 ppm; the significance of these effects has not been determined.[5] The suppression of dark adaptation and decrease in light sensitivity and the alteration of alpha brain wave patterns may affect performance to some unknown degree.[16,17] It is important to note that these effects occur at or below the human sensory threshold, before the subject becomes aware of the presence of the gas.

TABLE 1

Threshold Levels and Ranges for Significant Immediate Irritant and Neurologic Effects in Humans

Threshold Level or Range ppm SO_2	Effects	Reference
0.3-1	Lower limit of detectability. Neurologic effects, including elevation of optical chronaxy, suppression of dark adaptation, decreased light sensitivity, conditioning of electrocortical reflex, and disruption of alpha brain wave patterns.	5, 16-18
1-5	Range in which gas becomes detectable by taste and odor. Decreases in tidal volume and increases in respiratory and heart rates occur. Exposure by mouth breathing may cause coughing, throat irritation, increased salivation, bronchoconstriction. Dryness of throat and pharynx, bronchoconstriction, acrid taste, immediate coughing may occur with nasal breathing. Objectionable to some individuals.	10, 15, 19, 20
6.5-10	Definitely identifiable; causes nasal irritation, dryness of throat and pharynx, bronchoconstriction, coughing. May cause moderate to severe eye, nose, and chest irritation.	10, 18, 21-23
10-12	Objectionable to most human beings; least amount causing pain in nasal area and pharynx, conjunctival pain, rhinorrhea, severe discomfort.	4, 10
20-30	Least amount causing extreme irritation of upper respiratory tract, coughing, epistaxis, chest constriction, hemoptysis, severe bronchoconstriction, copious lacrimation, intense conjunctival pain. Extremely disagreeable.	22
30 and above	Least amount causing intense nasopharyngeal irritation, sneezing, coughing, epistaxis; few individuals exceed 15 minutes of exposure.	10

At 1 to 5 ppm, the gas will be detected by taste[18] and tidal volume will decrease, and there will be increases in the respiratory and heart rates which are not detected by the subject.[4] At 3 to 5 ppm, the gas will probably be detected by odor. If the subject is breathing by mouth (due to talking, exercise, or excitement), significant respiratory effects will occur at about 5 ppm. Coughing, throat and anterior chest irritation, increased salivation, and bronchoconstriction may occur,[15,19] probably adversely affecting performance but mild enough to be tolerable to most individuals. With exposure to 20 to 30 ppm, extreme upper respiratory tract irritation, coughing, epistaxis, chest constriction, hemoptysis, severe bronchoconstriction, intense conjunctival pain, and copious lacrimation will probably occur, with some individuals unwilling to tolerate exposure for more than 15 minutes.[7,22] These symptoms will probably impair performance.

2. Tolerance and Acclimation

Subjects may become tolerant to the irritant and mechanical pulmonary effects of sulfur dioxide at concentrations up to 25 ppm after several minutes of exposure.[15,19,22] Abrupt increases in concentration above a given level negate the adaptation to that level, until tolerance to the higher concentration occurs. Subjects gradually exposed to concentrations up to 25 ppm over the course of several hours will experience slight or no irritant or pulmonary symptoms, but a rapidly further increased concentration will require an adjustment period.[24] Since tolerance to irritant effects usually occurs within approximately 3 to 5 minutes after the onset of exposure, the duration of incapacitation or adverse performance may be sufficiently brief that the impairment of the efficiency of a soldier in training or combat may be negligible. At concentrations greater than 25 ppm, rapid tolerance probably will not occur.

The phenomenon of acclimation--gradual physiologic adjustment to sulfur dioxide after repeated, daily exposures--is less likely to occur with soldiers than with workers because of the erratic exposure schedules of the soldiers.

3. Interaction with Other Compounds

Interactions of sulfur dioxide with other compounds may modify its toxicity. Ammonia at sufficient concentrations has been shown to reduce both the irritant and pulmonary effects of sulfur dioxide.[25-27] This effect has been ascribed to ammonium sulfate formation.

Studies of the interactions of sulfur dioxide with aerosols of various compounds have produced conflicting results. Most data suggest, however, that exposure to combinations of sulfur dioxide with sodium chloride aerosols does not result in a potentiation of toxic effects.

4. Mode of Gas Inhalation

Differences will occur in irritant and respiratory effects of exposure to the same concentration of sulfur dioxide depending on the mode of breathing (i.e., mouth or nose). Mouth breathing (as compared with nose breathing) has been shown to both decrease the threshold concentration required for elicitation of effects and to increase the severity of effects.[15,19,22,28] The difference has been attributed to the different absorption capacities of the nose and mouth. Nasal absorption at physiologic flow rates has approached 99 percent of the inspired gas in both human and animal studies.[15,19,22,29] Mouth absorption of sulfur dioxide was significantly lower, allowing greater penetration of lower airways and stimulation of vagosympathetic receptors, thus inducing mechanical pulmonary changes and allowing the direct action of the gas on pulmonary tissue.

5. Effect of Gas Flow Rate

The mechanical flow rate of sulfur dioxide will be a significant factor in the genesis of sulfur dioxide-induced effects, especially with mouth breathing. Frank et al.[30] have shown that when sulfur dioxide at 1 and 10 ppm was delivered to dogs at flow rates of 3.5 and 35 liters per minute for 5 minutes, 99 percent nasal absorption resulted. Mouth absorption of the gas was also 99 percent at 1 and 10 ppm for 4.6 and 3.2 minutes, respectively, at a flow rate of 3.5 liters per minute. However, at 1 ppm for 1.6 minutes at a flow rate of 35 liters per minute, mouth absorption declined to 66 percent; thus, 34 percent of the inspired gas penetrated the lower airways. These findings are of practical significance in that the obligatory mouth breathing during talking, exercise, and excitement[31] and the consequently increased minute volumes likely to be exhibited by military personnel in training or combat situations may result in greater penetration of sulfur dioxide into the lower airways. The overall effect will be the reduction of threshold concentrations necessary to induce a variety of irritant, pulmonary, and neurologic responses and a possible increase in the severity of these effects at any given sulfur dioxide concentration.

The threshold level that will produce coughing, dryness of the throat, or throat irritation may be of some importance, as these symptoms may signify that sufficient though minor concentrations of the gas are penetrating into the lower respiratory passages and are capable of inducing irritant, respiratory, and central nervous system effects.

B. IRREVERSIBLE RESPIRATORY EFFECTS

No human studies have reported irreversible health effects except those following short, high-level exposures at unknown, extremely high sulfur dioxide concentrations.[7-11] In these instances, the effects have included immediate death, delayed deaths occurring up to 15 months after exposure, and, among the survivors, irreversible pulmonary damage.

Since the monkey resembles the human in biochemical adaptive mechanisms and responses to sulfur dioxide exposures, an example of animal exposure most applicable to humans was an accidental but nonlethal overdose of cynomolgus monkeys. Alarie et al.[12] continuously exposed one group of monkeys to sulfur dioxide at 4.7 ppm for 30 weeks, at which time the animals were accidentally exposed to between 200 and 1000 ppm for about 1 hour, but then were maintained on pure air for an additional 48 weeks. Before the accident, no adverse effects were observed. From the day of overdose, the monkeys experienced gradual and continuous deterioration of pulmonary functions. At autopsy, microscopic examination of the lungs revealed scattered foci of alveolar proteinosis, numerous alveolar macrophages, thickened alveolar walls with histiocytic infiltrates, moderate hyperplasia of bronchial epithelium, bronchiectasis, bronchiolectasis, and hepatocyte vacuolation. Since the animals demonstrated no adverse effects before the accident and other groups of exposed monkeys exhibited none of the above effects, the findings were attributed to the accidental, short, high-level exposure.

Because the actual sulfur dioxide level was not known, a conservative extrapolation of these data to humans suggests that exposure to sulfur dioxide at 200 ppm for 1 hour can produce extensive and irreversible damage in the pulmonary tract of the exposed individual.

IV. DISCUSSION

A. EXPOSURE LIMITS

In 1973, the American Conference of Governmental Industrial Hygienists (ACGIH) adopted a threshold limit value (TLV) of 5 ppm of sulfur dioxide as a time-weighted average (TWA).[1] The TLV was based on documentation indicating that below this level respiratory tract irritation would not occur in most workers, and that only minimal effects are to be expected in workers with continued sensitivity to SO_2 (suggesting that they may be incapable of acclimation). The TWA is thus derived from this value as a mean level of exposure for an 8-hour workday and is based on the assumption that chronic occupational exposure to this average level will not prove hazardous.

NIOSH[1] recommended that the current TLV of 5 ppm should be lowered to 2 ppm to prevent the changes in respiratory mechanics and the reduction of pulmonary function observed in both animals and humans at concentrations above 2.5 to 3 ppm.[2] In the 1978 notice of intended changes, ACGIH proposed a TLV of 2 ppm, which presumably will be adopted in 1980.[1]

The National Academy of Science National Research Council (NRC; unpublished) in 1966 recommended emergency exposure limits (EELs) for sulfur dioxide. No irreversible damage is expected to occur to the individual at the stated exposure limits if the incidence of exposure is rare (once or twice in a lifetime). The recommended EELs were:

- 30 ppm for 10 minutes
- 20 ppm for 30 minutes
- 10 ppm for 60 minutes
- 5 ppm for 24 hours.

The NRC is currently updating these recommendations and will probably release them in 1980.

The TLVs developed for use in the workplace generally would appear to have limited application in military situations, primarily because of differences in the anticipated conditions of exposure and in the objectives to be achieved by the creation of a standard. Unlike workers who may be exposed to fairly constant, low-level concentrations every working day, soldiers are likely to receive brief, intense exposures repeatedly over a period of 24 hours to several days, followed by periods of no exposure lasting days, weeks, or months. The main purpose of sulfur dioxide exposure standards for military application would be the prevention of casualties (i.e., immediate incapacitation or delayed health effects) and of effects that would impair immediate work efficiency and thus indirectly present a hazard to exposed personnel.

B. LETHALITY AND IRREVERSIBLE HEALTH EFFECTS

1. Accidental Occupational Exposure

Evidence of lethality and persistent health effects following accidental exposure to very high levels of sulfur dioxide is available in several case reports of occupational exposure.[7-11] In these cases, however, the unavailability of information on the actual sulfur dioxide concentrations and duration of exposure precludes any estimation of the lethal dose of sulfur dioxide for humans. These studies do, however, provide some data on the nature and sequence of changes in the clinical status of survivors, and also report postmortem findings in persons who have died as a result of these exposures. In these cases, gas concentration appeared to be far more important than the duration of exposure in determining the immediate postexposure outcome for the subject,[9] whereas both the concentration and duration of exposure influenced the delayed effects of the exposure. In addition to the usual irritant effects of sulfur dioxide inhalation (e.g., nose and throat irritation, sneezing, violent cough, and lacrimation), such high exposures in almost every instance resulted in either immediate death or delayed, irreversible symptoms, including death.

Immediate death (within 5 minutes of the beginning of exposure) was usually due to respiratory arrest and asphyxia, culminating in massive tracheobronchial mucosal necrosis and gross pulmonary edema, with no evidence of an inflammatory reaction.[9] The survivors of these accidents in most cases did not regain their preexposure pulmonary status.[7,11] Among the most common delayed irreversible findings were emphysema, bronchitis, and dyspnea at rest and on exertion, and often complete incapacitation. Other common findings included cough, wheezes, rales, hypoxemia, markedly abnormal pulmonary function tests, bronchiolitis obliterans, peribronchiolar fibrosis, and generally decreased small airway diameters. There was one instance of severe left main stem bronchial stenosis. Deaths from pulmonary infections (occurring 17 days to 16 months postexposure) have also been reported. The reduced resistance or enhanced susceptibility caused by sulfur dioxide may therefore ensue some days or months after what at first was considered to be a mild dose.[10]

Cases of sulfur dioxide-induced, irreversible asthma have been reported in previously healthy (but presumably predisposed) individuals after repeated exposures to sulfur dioxide.[29] In three cases cited, the asthma was debilitating, often responding temporarily to epinephrine injections, but apparently irreversible.

2. Human Experiments

Occasionally, human experiments have been conducted to determine the effects of extremely high concentrations of sulfur dioxide. Unfortunately, some of these data are from early studies and the reliability of the sulfur dioxide monitoring procedures cannot be assessed. In some studies, the method of sulfur dioxide determination was not stated. In

other studies, a nonspecific method (such as acidification with hydrogen peroxide and titration with base) was indicated as the method of analysis; however, no details were provided as to how the authors eliminated the presence of interfering substances such as other acid anhydrides, which would contribute to a false high reading. Thus, the reported effects may have occurred at slightly or even considerably lower concentrations than those stated. For example, in 1884, Ogata (cited by Charan et al.[9] and Greenwald[4]) reportedly exposed himself to sulfur dioxide at 554 ppm for 2 hours. Immediately upon exposure, he became extremely dyspneic and developed corneal clouding, both of which cleared after the exposure ended. He then exposed himself to 500 ppm of sulfur dioxide for a second time and found himself unable to breathe due to an irresistible urge to cough. Kisskalt in 1904 (cited by Greenwald[4]) reportedly exposed himself to 300 ppm of sulfur dioxide, which he was unable to breathe "for any length of time." He then exposed himself to 1500 ppm of sulfur dioxide, finding that he was unable to take even a single breath. Yamada in 1905 (cited in Greenwald[4]) reportedly exposed himself to sulfur dioxide at 140, 210, and 240 ppm, each for 30 minutes. Each exposure resulted in marked nasal irritation with sneezing and, at the two higher concentrations, eye irritation and increased lacrimation. Although these studies are of historical interest, none of these sulfur dioxide levels or total doses can be verified, and thus none can be considered as below the range of lethality or production of irreversible health effects in humans.

3. Animal Experiments

Most information on lethal concentrations of sulfur dioxide has been derived from animal studies.[4,14,38,40,41] While the qualitative response to sulfur dioxide in the respiratory system may be similar, the quantitative dose needed to produce similar changes varies between humans and other animals, with humans far more sensitive to sulfur dioxide.[4,42] Of the species tested, rats were found to be the most resistant to sulfur dioxide exposures, followed in order by rabbits, rhesus monkeys, guinea pigs, mice, and humans.[4,13,32]

Lethality data derived from various animal studies (given in Table 2) are of limited use in predicting lethal levels of sulfur dioxide for short-term exposure of humans. Many of these studies used extremely long exposure times to establish LCt_{50}s at comparatively low sulfur dioxide concentrations (e.g., 112 ppm for 113 hours in guinea pigs,[21] 150 ppm for 847 hours in mice[32]). Short-term exposures of 1 hour or less may provide more relevant data for the purposes of predicting the results of acute human exposure.

Potentially useful information is available from a study by Alarie et al.[12] in which nine cynomolgus monkeys that had been exposed to sulfur dioxide at 4.69 ppm for 30 weeks were accidentally exposed to sulfur dioxide at between 200 and 1000 ppm for 1 hour. For the remaining 48 weeks of the study, these animals remained on ambient air. While none of these monkeys died, the histopathologic findings in the respiratory tract were similar to those observed in humans accidentally exposed to high

TABLE 2

Lethality Data From Animal Studies

Species	SO_2 Concentration, ppm	Time	Lethality Criterion	Reference
Mice	1948	9.8 min	LCt_{50}	14
	1375	37.5 min	LCt_{50}	
	1337	10 min	LCt_{50}	
	878.6	217 min	LCt_{50}	
	611	59 min	LCt_{50}	
	1222	222 min	CtD_{50}	
	1184	39 min	MCt_{50}	
	1108	366 min	CtD_{50}	
	993	[illegible] min	CtD_{50}	
	993	108 min	CtD_{50}	
	916.8	258 min	80%[a]	
	916.8	75 min	MCtD	
	764	20 min	LC[b]	
	611	288 min	MCtD	
	611	300 min	LC[b]	
	1400	175 min	100% mortality	
Mice	3000	30 min	LCt_{50}	6
Mice	130	24 hours	LCt_{50}	18
	340	6 hours	LCt_{50}	
	610	1 hour	LCt_{50}	
	1350	10 min	LCt_{50}	
Mice	150	847 hours	LCt_{50}	32
	1000	4 hours	LCt_{50}	
Guinea Pigs	130	154 hours	LCt_{50}	
	1000	20 hours	LCt_{50}	
Guinea Pigs	3910	76 min	100% lethality	38
	1270	76 min	LCt_{50}	
Mice	1070	730 min	100% lethality	
	560	730 min	LCt_{50}	
Guinea Pigs	112	113 hours	LCt_{50}	21
Rats	>600	<1 min	100% lethality	13
Rats	400	5 hours/day	3 dead at 5 hours (10%)	
		5 days/week	22 dead at 7 days (73%)	
			25 dead at 12-15 days (83%)	
Syrian Hamsters	400	5 hours/day	1 dead at 45 min	13
			all dead at 6.2 hours	

TABLE 2 (cont'd)

Lethality Data From Animal Studies

Species	SO_2 Concentration, ppm	Time	Lethality Criterion	Reference
Syrian Hamsters	400[c]	5 hours/day 5 days/week	No death in 35 days	13
Rats	567	6 hours/day 5 days/week	87% dead in 12 days	43
Mice, Rats, Rabbits	50	6 hours/day for 30 days	100% lethality in mice and rabbits	4
Guinea Pigs	312	112 hours	LCt_{50}	4
Cats, Dogs, Rabbits	800-1000	20-30 min	LC[b]	40
	500-600	60 min	LC[b]	

[a] Nine died during exposure, four more within the 2-week inspection period.

[b] Probably 100% mortality, details unknown.

[c] Animals were exposed in gradual increments over 4 days, starting with 250 ppm on day 1.

doses of sulfur dioxide, with inflammation, bronchiectasis, bronchiolectasis, and alveolitis, but, unlike humans, little fibrosis.

Laskin et al.[43] intermittently exposed rats to sulfur dioxide at 10, 51, 105, and 567 ppm for 6 hours per day, 5 days per week for 113, 112, 22, and 12 exposure days, respectively. All exposure-induced mortality was observed within the first 3 weeks. At day 12, incidences ranged from 4.7 percent at 10 ppm to 87.2 percent at 567 ppm.

Gross pulmonary lesions and clinical symptoms were absent at 10 ppm and most marked at 567 ppm, with spontaneously dying animals exhibiting degenerative changes including congestion, edema, loss of cilia in trachea and bronchi and cell hyperplasia and stratification. Serial sacrifice studies revealed tracheitis in animals at all exposure levels and high incidences of bronchitis, congestion, and pneumonia at 105 and 567 ppm. Regenerative changes in the trachea occurred at all exposure levels but were most extensive at 105 and 567 ppm. Rats exposed to 105 ppm had regenerative hyperplasia and early metaplasia after only 4 days of exposure. However, the mucosal changes were reversible within 2 weeks after the cessation of exposure.

Asmundsson et al.[13] found that Syrian hamsters exposed to sulfur dioxide at 400 ppm for 5 hours per day all developed respiratory distress, with one animal dying within the first 45 minutes of exposure and the longest surviving animal dying after 6.3 total exposure hours. This suggests that 400 ppm is within the range of lethality for humans since hamsters are a species reportedly less sensitive to sulfur dioxide toxicity than are humans.[4,13,32,42] Pathologic changes reported at necropsy of these hamsters included marked vacuolar degeneration of tracheal epithelium and large areas of necrosis and hemorrhage. Bronchi showed similar but less extensive damage, and bronchioles showed patchy vacuolation and degeneration of cells without extensive damage.

However, when a second group of hamsters was exposed to gradually increasing sulfur dioxide concentrations (from 250 ppm on the first day to 400 ppm by the fourth day) for 5 hours per day, 5 days per week for a total of 35 exposure hours, no spontaneous deaths occurred. Pathologic changes seen at necropsy were similar to, but not as extensive as, those observed in animals abruptly exposed to 400 ppm.

A similar strategy was used in a study by Islam et al.[44] Two-thirds of a group of dogs exposed to sulfur dioxide at 500 ppm died almost immediately. The authors then attempted to expose dogs to this level of gas by gradual increments starting at 300 ppm for 2 hours per day. Dosages and times of exposure were gradually increased and were interspersed with two periods (10 days and 3 weeks) of nonexposure. The final exposure level was 500 ppm for 8 hours per day, 5 days per week for 60 days (235 exposure hours). The total exposure time was 433.5 hours in 160 days.

At 300 ppm, upon initial exposure each day, the dogs vomited, coughed, and had excessive white foamy secretions in the mouth and nose. Their eyes were extremely irritated, with copious secretions and conjunctional infection that continued during exposure. During the 10-day midexposure recovery period, nasal secretions, coughing, and eye symptoms continued. At 400 ppm, oral, nasal, and eye secretions initially increased. During the 3-week midexposure recovery period, the dogs had piping respiratory sounds and continued nasal secretion but a reduction in corneal inflammation.

At 500 ppm, corneal inflammation and oral, nasal, and eye secretions immediately increased, subsiding somewhat after the first week. All animals developed rales after 1 week of exposure. Following exposure, rales were heard in one-third of the dogs; mouth, nose, and eye secretions visibly improved, as did the corneal inflammation. The expulsive cough continued as intensely as during exposure. Histopathologic examination revealed an increase in goblet cells in the trachea, and a slight increase in the number of serous secretory cells in the main and lobar bronchi (but not on the bronchioles), and an increase in intraductular material in the bronchi. Mild interalveolar fibrosis without inflammatory cell infiltration was observed.

The authors suggested that the intermittency of the exposure to these extremely high levels probably allowed for partial repair of injured tissues. While the histopathologic examination (performed 2 weeks after the final exposure) revealed what the authors considered to be minor effects, previous human[7-11] and animal studies[12,13] suggest that these lesions were irreversible and that with time progressive deterioration of lung function would ensue.

Bitron and Aharonson[14] measured the mortality rates and times to death of mice following a single exposure to sulfur dioxide. This study provided these estimates of the Lt_{50} and the LCt_{50} mice exposed to high levels of sulfur dioxide:

- At 900 ppm the Lt_{50} was about 200 minutes. Therefore, the LCt_{50} was about 3000 ppm·hours.
- At 1400 ppm, the Lt_{50} was about 38 minutes. Therefore, the LCt_{50} was about 887 ppm·hours.
- At 1900 ppm the Lt_{50} was about 10 minutes. Therefore, the LCt_{50} was about 317 ppm·hours.

The number of delayed deaths after exposure was also recorded at each of these dosage levels in order to determine a median time of death (MTD, time within which one-half of the animals died during postexposure only). The following estimates were provided:

- At 900 ppm for 200 minutes (3000 ppm·hours), the MTD was 7 days.

- At 1400 ppm for 38 minutes (887 ppm hours), the MTD was 9 days.
- At 1900 ppm for 10 minutes (317 ppm hours), the MTD was 10 days.

Because mice are reportedly less sensitive to the effects of sulfur dioxide than are humans, these data help establish that exposure to sulfur dioxide at 900 ppm for 200 minutes is well within the range of immediate and slightly delayed lethality for humans.

Some studies indicate that exposure to relatively low levels of SO_2 may have irreversible effects. For example, O'Donoghue and Graesser[45] exposed guinea pigs to sulfur dioxide at 5, 10, 20, and 40 ppm for a single, continuous 8-hour exposure. All concentrations resulted in evidence of irritation. At 5 ppm, there was mild eye irritation and increased salivation; at 10 ppm and higher, there was increased nasal secretion and salivation and altered respiration. All of these effects were reversible. However, animals exposed to 40 ppm exhibited hemorrhage and emphysematous changes in the lungs at autopsy 24 hours and 7 days postexposure. At 158 days postexposure, both animals exposed to 40 ppm and one of two animals exposed to 20 ppm showed pulmonary fibrosis.

Ball et al.[39] continuously exposed rats to sulfur dioxide at 0, 2, 4, 8, 16, and 32 ppm for 8 to 16 months. Exposed rats developed wheezing, eye opacities, fur loss, scaly tails, and increased levels of blood neutrophils and hemoglobin directly proportional to the concentration and duration of exposure. At 8 months of exposure, 100 percent of the control rats, 93 percent of rats exposed to 1 to 16 ppm and 82 percent of rats exposed to 32 ppm of the gas remained alive; at 12 months, the survival rates for these groups were 91, 84, and 58 percent, respectively, and at 16 months, 75, 68, and 44 percent, respectively. Pathologic effects and survival rates were concentration-time related.

Most of these studies provide data on the effects of exposure to extremely high levels of sulfur dioxide that may never be encountered in a training or combat situation. However, the results of the study by O'Donoghue and Graesser[45] suggest that irreversible changes in pulmonary tissue may occur with a single continuous 8-hour exposure to sulfur dioxide at levels as low as 20 ppm. Further investigation of histopathologic changes at this and similar levels is warranted.

C. IMMEDIATE, REVERSIBLE IRRITANT EFFECTS

The immediate irritant effects of sulfur dioxide inhalation are important because, in addition to their potential association with adverse long-term health effects, they are able to produce immediate, adverse changes in performance and behavior. Reported irritant responses in humans at various concentrations of sulfur dioxide are presented in Table 3. In certain instances, data are contradictory; for example, minimum concentrations at which coughing was induced included 5 ppm,[46] 10 ppm[4], and 20 ppm.[10] In most instances, the lower values are the most recent and probably more accurate than higher values, which are the older values reported by Henderson and Haggard[47] as cited by

Gordon.[10] As mentioned previously, in many early studies, either the method of sulfur dioxide analysis was not stated or specific details were not provided as to how interfering substances were removed to prevent a high reading in a nonspecific method. Differences in values may also be due to the subjective interpretation of the onset of a symptom; what may be irritating to one individual may cause only negligible discomfort in another and thus not be reported. Motivation also varies among test subjects, and some persons may be willing to endure certain discomforts for a longer period. All values should therefore be viewed as rough estimates of concentrations causing particular effects.

1. Epidemiologic Data

In general, the irritant effects of sulfur dioxide are proportional to the gas concentration in causing discomfort[22] and are immediate in onset, occurring within the first few breaths after exposure begins. In a 1932 epidemiologic study, Kehoe et al.[24] compared the responses given to questionnaires by 100 unexposed workers to the responses of 100 workers chronically exposed to sulfur dioxide at reported concentrations of 5 to 70 ppm with an average daily range of 20 to 30 ppm. Because no method of sulfur dioxide analysis was stated in this study, the accuracy of these exposure levels cannot be assessed and these data will be used only to investigate the qualitative effects of sulfur dioxide exposure, including type of symptoms, order of onset, and development of tolerance and acclimation to the irritant effects of sulfur dioxide exposure. Kehoe et al. reported that new workers, upon initial exposure to this work environment, experienced irritant symptoms in a specific sequence: upper respiratory tract irritation, coughing, epistaxis, constriction of the chest, and hemoptysis. These symptoms were immediate in onset; most developed within minutes to hours of the start of the exposure period. The severity, sequence, and time of onset of symptoms were determined by the exposure concentration. Other authors have reported that irritant symptoms, induced by concentrations as low as 4.5 ppm, followed no specific order of appearance.[19]

2. Human Experiments

a. Physiologic Response

Sulfur dioxide can be detected by humans as a foreign substance in the air at approximately 0.3 to 1 ppm (probably by taste)[18,41]; the least detectable odor occurs at approximately 3 to 5 ppm[4]; and 5 to 10 ppm inhaled nasally normally causes irritation of nasopharyngeal mucosa and coughing on initial exposure.[4,20,41] Exposure to sulfur dioxide at low to moderate concentrations (1 to 25 ppm) can induce tolerance to the irritant symptoms within 3 to 4 minutes of exposure, making it possible to work without discomfort for long periods of time.[15,19,20,22,41,48,49]

Hine et al.[23] found that volunteers exposed to various pollutants, including sulfur dioxide, exhibited various degrees of irritant responses, including eye irritation, nose irritation, pulmonary discomfort,

changes in olfactory recognition, and central nervous system effects (e.g., headache, nausea, vertigo). Of the different substances tested, exposure to 9.1 ppm of sulfur dioxide for 6 minutes elicited the greatest degree of subjective discomfort, rates between moderate and severe, and decreasing in severity during the time of exposure. Frank[15] reported that exposure to sulfur dioxide at 13 ppm (range: 10 to 16 ppm) resulted in immediate coughing, throat and upper chest irritation, and increased salivation, all of which subsided within the first few minutes of exposure. However, in this as in other studies,[19,20] mechanical pulmonary effects, such as bronchoconstriction, were still intensifying as the irritant effects faded. Anderson et al.[22] reported almost intolerable discomfort, coughing, and rhinorrhea in subjects exposed to 25 ppm, with all symptoms subsiding within a few minutes of the start of exposure and remaining decreased even while exposure continued.

From the military standpoint, it is of interest that a variety of irritant symptoms presented in Table 3 occur at or below the TLV of 5 ppm for sulfur dioxide. Of potential concern is the narrow margin between the detection of the gas by its odor at the 3 to 5 ppm concentration and the onset of irritant symptoms (by nasal breathing) at 5 ppm to 6.5 ppm, sufficient to impair the performance capabilities of an exposed person (e.g., dry throat and pharynx, nasal irritation, acrid taste, bronchoconstriction, objectionable odor, and immediate coughing).

b. Effect of Motivation

The motivation or psychologic "staying power" of the exposed individual has been addressed as a factor that may affect the individual's response to sulfur dioxide, both in relation to general discomfort and toleration of irritant symptoms. In a study by Anderson et al.[22] 15 subjects were exposed gradually over a 1-hour period to SO_2 at concentrations of 1, 5, and 25 ppm for up to 8 hours per day. Two subjects apparently remained unaffected, according to the self-rating system employed in the study. For the other 13 subjects, the reported discomfort was proportional to the increases in gas concentration, and was accompanied by increases in airway resistance and decreases in mucous flow rates proportional to the concentration and duration of exposure. With exposure to 25 ppm, these 13 subjects reported various irritant symptoms including nasopharyngeal pain and dryness, rhinorrhea, conjunctival pain, and coughing. Even the experimenters reported intolerable discomfort at this concentration. In contrast, the lack of any display or verbal expression of discomfort by the other two subjects when exposed to 25 ppm of sulfur dioxide appeared to be unusual, especially in light of their changes in mucous flow rates and airway resistance. Because they played cards while others reported intolerable discomfort, the authors suggested that motivation of some sort, rather than simply physical tolerance, was a factor in their behavior.

c. Mode of Intake

An important factor regarding irritant symptoms and other effects (e.g., pulmonary clearance, sulfur dioxide absorption) of sulfur dioxide exposure is the mode of intake of the gas, i.e., nasal breathing or oral

Table 3

Reported Human Physiologic Responses to Various Threshold Concentrations of Sulfur Dioxide

Effects	Threshold Levels, ppm	Reference
Least amount causing conditioning of electrocortical reflex	0.23	5
Least amount detectable (probably in taste)	0.3 to 1	18
Least amount causing suppression of dark adaptation, decreased light sensitivity, and disruption of alpha brain wave patterns	0.35	5, 16
Least amount causing elevation of optical chronaxy	0.58	5
Least amount causing (unnoticed) increase in heart rate and respiratory rate and decrease in tidal volume	1 to 5	4
Least detectable odor	3 to 5	10
Least amount causing coughing, throat irritation, increased salivation, bronchoconstriction (during quiet mouth breathing)	4.5	19
Least amount causing throat and upper chest irritation, coughing, bronchoconstriction (during quiet mouth breathing)	4 to 6	15
Least amount causing dryness of throat and pharynx, bronchoconstriction, acrid taste, immediate coughing; objectionable to some individuals	5	4, 20
Least amount causing nasal irritation	6.5	4
Least amount definitely identifiable	6 to 8	4
Least amount causing immediate irritation to throat and nose, objectionable to most humans	6 to 12	10, 18
Least amount causing moderate to severe eye, nose, and chest irritation	9.1	23
Least amount causing coughing	10	4
Least amount causing coughing	20	10
Least amount causing pain in nasal area and pharynx, conjuctival pain, rhinorrhea, severe discomfort, coughing, throat irritation	10	4, 21, 22
Maximum allowable concentrations (MAC) for prolonged exposure	10	

Table 3

Reported Human Physiologic Responses to Various Threshold Concentrations of Sulfur Dioxide (Cont.)

Effects	Threshold Levels, ppm	Reference
Least amount causing immediate irritation to eyes	20	10
Least amount causing intense eye, nose, and throat irritation, epistaxis; well above bronchoconstrictive levels, extremely disagreeable	20	4
Least amount causing extreme irritation of upper respiratory tract, coughing, epistaxis, chest constriction, hemoptysis	20 to 30	24
Least amount causing intense nasopharyngeal irritation, sneezing, coughing, epistaxis; no individual exceeded 15-minute exposure	30	4, 24
Maximum allowable concentrations (MAC) for short exposures (0.5 to 1 hour)	50 to 100	
Least amount causing extremely severe irritation of respiratory mucosa, inhibition of respiration, glottal spasm	100	40
Amount causing inability to breathe for any length of time	300	4
Dangerous for even short exposures (0.5 hours or less)	400 to 500	10
Amount resulting in inability to take full breaths, irrisistable urge to cough, reversible corneal clouding	500 to 514	10
Amount resulting in inability to take full breath; insupportable of human life	1500	4
Irritating to moist areas of skin	10,000	18

breathing. The sulfur dioxide concentrations and the respective irritant symptoms, discussed previously, occurred in association with quiet nasal breathing, except where indicated, as the mode of gas inhalation. Humans normally breathe through the nose but switch to mouth breathing during talking [30,31] and exercise.[19,30,31,50] In a combat or training situation, an individual is likely to use an oral breathing pattern and to develop an increased respiratory rate, due to both psychologic factors, such as excitement and stress, and physiologic factors, including increased physical exertion.[19,30,31,50] In addition, sulfur dioxide itself causes an increase in nasal airway resistance, which further increases the work of nasal breathing and thus increases the tendency toward oral breathing.[19,20,30] The high flow rates during mouth breathing result in increased lower airway exposure (out of proportion to the minute ventilation or airflow) and hence relatively greater irritant effects in the lung (as well as pulmonary and other effects) compared to the effects of nasal breathing.[30] In addition, mouth breathing bypasses the nasal absorption mechanism by which 99 percent of the inspired gas is absorbed by the nasal mucosa.[15,30,51] Thus, with oral breathing, more sulfur dioxide is available to penetrate the lower airways. Frank et al.[19] reported that quiet mouth breathing of 4.5 ppm of sulfur dioxide caused the immediate onset of coughing, throat irritation, anterior chest irritation, and increased salivation. All of the symptoms appeared within 1 to 2 minutes of exposure to the gas, following no specific order of onset, and subsided before the end of exposure at a time when pulmonary mechanical effects were maximal. The heightened response of the subjects to this concentration of sulfur dioxide mimicked that observed in dogs breathing the gas through a tracheostomy.[19,52] The response was attributed to a bypassing of the nasal mucosa and thus a decrease in nasal absorption[19] and subsequently greater exposure of the lower airways to the gas.[30] Frank[15] reported that subjects exposed to 15 and 29 ppm of sulfur dioxide for up to 25 minutes by quiet mouth breathing experienced coughing and throat and upper chest irritation within the first few breaths of the gas. In contrast, subjects exposed to the gas during quiet nasal breathing rarely coughed or reported chest irritation. The author concluded from the extent and severity of the irritant symptoms that the sulfur dioxide appeared to penetrate further along the airways when inhaled by mouth rather than by nose.

The findings of Frank et al.[30] have also suggested that in humans the increased rate of airflow during mouth breathing was as important as gas concentration in determining the degree of airway penetration in relation to irritant symptoms. Therefore, the obligatory mouth breathing and increased rate of airflow during exercise and heavy labor are likely to result in increased lower airway exposure that is out of proportion to the increase in minute ventilation, resulting in the onset of symptoms at a lower gas concentration.[30]

The irritant effects of repeated, short (less than 1 hour), high-level (5 ppm up to about 29 ppm) exposures to sulfur dioxide, by both nose and mouth breathing, subside usually before or upon termination of exposure and appear to be completely reversible.[4,15,19,20,22,23,48,49]

3. Animal Studies

Animal exposures resulting in reversible irritant effects are of questionable value, given species variability and the abundance of human data. In addition, some of these are early studies and the accuracy of the reported sulfur dioxide concentrations cannot be assessed. For example, in 1908, Ronzani (cited in Greenwald[4]) exposed rabbits, guinea pigs, and pigeons to sulfur dioxide at reported concentrations of 50 to 500 ppm for 6 to 7 hours per day. Animals exposed to 500 ppm immediately experienced restlessness, sneezing, and lacrimation, with symptoms subsiding by the end of the second exposure week. Animals exposed to 50 ppm showed no obvious ill effects. Ogata (cited in Greenwald[4]) in 1884 reportedly exposed rabbits, guinea pigs, and mice to 400 ppm for 4 hours, resulting in dyspnea and corneal clouding, which were reversible after the exposure ended. Kisskalt (cited in Greenwald[4]) in 1904 exposed rabbits to sulfur dioxide at 730 ppm for 9 hours, during which time the animals remained motionless and developed corneal clouding. Upon removal from the exposure chamber, they regained mobility and corneal clouding disappeared by the following day. Follow-up observations at 3 weeks revealed healthy animals. However, exposure of rabbits to 170 ppm for 9 hours per day for 8 days resulted in irritant symptoms plus the death of half of the exposed animals.

These animal studies generally suggest that repeated high-level long-term exposures (longer than 1 hour per exposure) are more dangerous than single or repeated, high-level short-term exposures over a shorter time span[4] (except at lethal sulfur dioxide concentrations). However, because the extremely high levels of sulfur dioxide reported in these studies cannot be assumed to be accurate, this can only be viewed as preliminary data, subject to verification by current literature. In more recent studies, reversible responses of various kinds, although mainly irritant, have been observed as a result of sulfur dioxide exposure. Giddens and Fairchild[53] exposed disease-free mice and mice with upper respiratory tract disease to sulfur dioxide at 10 ppm for up to 72 hours. The earliest lesions, observed at 24 hours in the nasomaxillary turbinates, consisted of edema, necrosis and desquamation of respiratory and olfactory epithelia. The diseased mice had more severe lesions than the disease-free mice, and both groups demonstrated more severe lesions in the nasomaxillary turbinates than in the remainder of the respiratory tract. Even after 72 hours of exposure, the trachea and lungs of both groups were practically free of lesions. The observed effects were completely reversible.

Johnson et al.[54] continuously exposed mice to sulfur dioxide at 40 ppm for 4 to 11 days and 33 to 35 days. Mice exposed for 4 to 11 days demonstrated statistically significant decreases in food intake, water intake, and body weight (p 0.01 for all variables) compared to control values. Postexposure observation for 12 days revealed an overcompensation in food and water intake, with body weights eventually approaching control values. The effects were clearly reversible. Mice exposed for 33 to 35 days initially experienced the identical significant declines in food intake, water intake, and body weight (p 0.01) compared to controls. However, with continued exposure their intake of food and water

approached control values, with only body weight and oxygen consumption remaining significantly decreased compared to control values ($p < 0.01$). The animals appeared to be in poor condition with dry, dull eyes, irregular breathing, sneezing, sniffing, and arched posture. Three of these animals died and histopathologic examination of their respiratory tracts revealed accumulated mucus, cellular debris, numerous leukocytes, thick and poorly organized stratified squamous epithelium, and thinning of nasal turbinate bones with several instances of their complete dissolution. No gross changes were observed in the lungs. Postexposure observation of the surviving animals for 34 days again revealed a compensatory increase in food and water intake that began almost immediately. On day 32 post- exposure, all values, including oxygen consumption, were similar to control levels, with food intake remaining higher ($p < 0.05$). Despite the extensive upper respiratory tract damage during exposure, at 32 days postexposure, no gross or microscopic effects in the upper respiratory tract were observed; the exposed and control animals appeared very similar. Apparently, the postexposure period was sufficient for repair of at least some of the damage in the upper respiratory tract.

The results of these two studies suggest that in mice, with exposure to sulfur dioxide at some level between 10 and 40 ppm, irreversible histopathologic changes may begin to occur in the respiratory tract.

Alarie et al.[55] continuously exposed guinea pigs to sulfur dioxide at 0.13, 1, and 5.7 ppm for 12 months. Tidal volume, respiratory rate, minute volume, dynamic compliance, and pulmonary flow resistance values indicated no detrimental lung changes attributable to sulfur dioxide exposure, nor were hematologic parameters, clinical chemistry, body weight, growth, and survival rates adversely affected. Microscopic examination revealed that animals exposed to 5.7 ppm had a lower incidence and severity of spontaneous disease than that seen in controls. However, microscopic examination of the liver revealed an increase in hepatocyte size and cytoplasmic vacuolation in animals exposed to 5.7 ppm. The reversibility of these hepatic changes was not addressed.

D. NEUROLOGIC EFFECTS

Central nervous system effects due to sulfur dioxide exposure may alter the immediate behavior and job performance of an individual. Disorienting responses such as vertigo or nausea can temporarily but totally incapacitate some persons. Interference with certain sensory functions, such as sight or hearing, can result in impaired responses to occupational stimuli. Such aberrations, however slight, assume magnified importance when they occur during an intrinsically hazardous activity such as driving, the operation of heavy machinery, or the use of weapons.

1. Human Experiments

Bushtueva et al.[17] determined the perception threshold for odor and mucosal irritation for sulfur dioxide and its oxidation product, sulfuric acid. These levels were found to be 0.62 to 1.1 ppm for sulfur dioxide and 0.15 to 0.21 ppm for sulfuric acid. Optical chronaxy (the

time interval between the appearance of light and the optical registration of light) was not altered by concentrations below the sensory threshold of either toxicant. However, 9-minute exposures to either sulfur dioxide or sulfuric acid at concentrations slightly above the sensory threshold consistently elicited increases in optical chronaxy. This implies that a person exposed to sulfur dioxide at a concentration just below the present TLV of 5 ppm would take more time to perceive a given intensity of light than would a nonexposed individual.

Lewis et al.[5] reviewed the sensory and central nervous system effects of sulfur dioxide. Exposures to combinations of sulfur dioxide and sulfuric acid resulted in an additive increase in optical chronaxy. The information on optical chronaxy was further substantiated by studies on dark adaptation or eye sensitivity to light. Subthreshold concentrations had no effect on dark adaption, but slightly higher concentrations reduced dark adaptation activity in the visual cortex after 20 minutes' exposure. This response was diphasic, as some subjects noted a postexposure return to normal, followed by a markedly depressed light sensitivity 50 to 60 minutes later.

Alpha rhythms were monitored by electroencephalography in subjects exposed to sulfur dioxide and sulfuric acid. Alpha waves are the regular brain waves characteristic of the awake and relaxed (as opposed to excited or drowsy) state. Desynchronization of alpha rhythms occurred only when the sensory threshold concentrations of sulfur dioxide or sulfuric acid were used and lasted only for 1 to 2 seconds. Concentrations below the sensory threshold did not appear to alter the central nervous system signals. At higher concentrations, the crude subjective indices of odor perception and mucosal irritation correlated well with the onset of these alterations in the central nervous system. Apparently, trigeminal nerve excitation (involved in mucosal membrane irritation) and olfactory receptor excitation (involved in odor perception) not only reflexively affect alterations in alpha rhythms, but are also associated with optical chronaxy and dark adaptation. Subjective responses therefore appear to be as valid as objective indices in assessing the cortical effects of sulfur dioxide and sulfuric acid.

Electrocortical conditioned reflexes were investigated using desynchronization of alpha rhythms as the index. Nonexposed individuals experienced alpha rhythm desynchronization only upon exposure to light. Other subjects were first repeatly exposed to the light plus the irritants at subthreshold levels; when later exposed solely to subthreshold levels of the irritants, they displayed desynchronization of their alpha rhythms. The concentrations at which this phenomenon occcurred were 0.23 ppm of sulfur dioxide, 0.10 ppm of sulfuric acid, and combinations of the two irritants at either 0.19 ppm of sulfur dioxide with 0.04 ppm of sulfuric acid or 0.09 ppm of sulfur dioxide with 0.07 ppm of sulfuric acid. These levels suggest an additive effect. The complex neurologic process resulting in disruption of cortical rhythm was therefore initiated by a level below the threshold of sensory perception (Table 4).

TABLE 4.

Threshold Concentrations Required For Sensory Response (ppm)[a]

	SO_2	H_2SO_4	$SO_2 + H_2SO_4$	
Perception of Odor and Irritation of Mucosa	0.62-1.1	0.15-0.21	0.19	0.07
Supression of Dark Adaption and Decreased Light Sensitivity	0.35	0.15-0.18	0.19	0.07
Elevation of Optical Chronaxy	0.58	0.18	0.46	0.15
Disruption of Alpha Rhythms	0.35	0.15	0.19	0.07
Conditioning of Electrocortical Reflex	0.23	0.10	0.19 (0.10	0.04 or 0.07)

[a] Table data from Lewis et al.[5] and Xintaris and Johnson.[16]

The implications of central nervous system effects at sulfur dioxide concentrations well below the TLV of 5 ppm may be of considerable importance. The decreased light sensitivity and suppression of dark adaptation may be of sufficient magnitude to impair the efficiency of individuals involved in night maneuvers. However, whether these central nervous system alterations are sufficient to significantly impair visual responses is unknown. Although increased concentrations of the irritants further increased optical chronaxy and decreased light sensitivity and dark adaptation,[5] it is not known whether this relationship continues at much higher levels of sulfur dioxide. In addition, there is a very narrow safety margin between the concentrations at which the gas is perceived and those at which objective central nervous system alterations occur; thus the exposed person would have no warning of impending visual effects.

2. Epidemiologic Data

In a study by Kehoe et al.,[24] workers chronically exposed to sulfur dioxide at reported concentrations averaging 20 to 30 ppm demonstrated increased fatigability, abnormal deep tendon reflexes, shortness of breath, altered sense of taste and smell, and general irritability. Workers' reactions to exposure varied from sluggishness to hyperactivity. However, these symptoms were not the type associated with localized lesions of the central nervous system and the authors concluded that the central nervous system changes signified a variation of generalized central nervous system irritability.

E. IMMEDIATE RESPIRATORY EFFECTS

The immediate respiratory effects of exposure to sulfur dioxide range from minor impairment of pulmonary function to irreversible pulmonary impairment and death.[56] (Lethality and irreversible health effects are discussed in Section B.) The occurrence of these effects is largely but not totally dependent on gas concentration and duration of exposure.

1. Epidemiologic Data

Adverse subjective changes in respiratory capacity upon initial exposure to sulfur dioxide at reported levels of 20 to 30 ppm have been described by Kehoe et al.[24] in a 1932 epidemiologic survey of sulfur dioxide-exposed workers. In addition to the irritant effects observed by new workers, respiratory effects such as a feeling of chest constriction (possibly representing bronchoconstriction), coughing, and hemoptysis were also reported. Long-term, repeated, intermittent exposures resulted in increased fatigability and shortness of breath upon exertion, with the reported occurrence of each symptom positively correlated with the frequency of exposure.

2. Experimental Data

Immediate changes in pulmonary function as a result of sulfur dioxide exposure have been observed in a number of laboratory studies in humans and in various animal species. The most common parameter used has been changes in airway resistance, especially pulmonary flow resistance (PFR). The differential effects of oral and nasal exposure to sulfur dioxide have also been investigated, as have been the results of exposure to sulfur dioxide in combination with aerosols of distilled water or saline, or with ammonia gas. Some studies have attempted to elucidate the mechanism of bronchoconstriction that results from exposure to sulfur dioxide.

a. Effects on Immediate Respiratory Capacity

(1) Human Experiments

Frank[15] exposed human subjects to sulfur dioxide by mouth breathing in a body plethysmograph. The exposures resulted in average increases in PFR of 0 percent at 1 ppm, 39 percent at 5 ppm, and 72 percent at 13 ppm, for 10-minute exposures. The changes in PFR occurred within the first minute of exposure and were maximal after 5 to 10 minutes. It was reported that there was no observable relationship between the duration or amount of cigarette smoking and changes in PFR in response to sulfur dioxide exposure. Irritant symptoms elicited at 4.5 ppm and higher levels of sulfur dioxide appeared within the first few breaths (1 to 2 minutes), subsiding as the bronchoconstrictive effects were still intensifying. In some subjects, the 5 and 13 ppm exposures were prolonged to 30 minutes. Although the PFR values remained elevated above control values during the entire sulfur dioxide exposure, they diminished from their peak levels after the first 10 minutes.

Sim and Pattle[25] were unable to demonstrate any changes in pulmonary flow resistance in human subjects breathing sulfur dioxide by nose during repeated (no more than twice a week), intermittent (at least 24 hours between exposures) exposures of 30.8 ppm or less for 10 minutes by mask and 5.1 ppm or less for 60 minutes in a chamber. Occasionally, 20 percent increases in PFR were observed, accompanied by chest irritation and moist rales, bilaterally and anteriorly over the large bronchi. One-half of the subjects exposed to sulfur dioxide at concentrations of 51.2 ppm or more for 10 minutes and 8.5 ppm or more for 60 minutes demonstrated significantly increased PFR values (actual values were not given). The greatest increases in PFR occurred within the first 10 minutes of exposure, with little further change after 1 hour. Irritant symptoms of lacrimation and rhinorrhea occurred, and the most frequent clinical observation at these dosages was high-pitched musical rales, with prolonged expiration time. Moist rales were also heard in the peripheral lung fields in subjects receiving prolonged exposures and anteriorly over large bronchi in those receiving smaller doses or shorter exposures.

The discrepancy between the findings of Frank[15] and Sim and Pattle[25] regarding the concentrations of sulfur dioxide eliciting changes in PFR may be attributed to differences in the mode of breathing in these two studies. As described previously, the nasal breathing used in Sim and Pattle's study would have resulted in a 95 to 99 percent uptake of inhaled sulfur dioxide by the nasopharyngeal mucosa,[25] whereas the oral breathing used in Frank's study would have bypassed this absorptive mechanism and resulted in greater exposure of the lower airways to the gas.[30]

Melville's[28] study on airway resistance contradicted some of the findings and conclusions of both Frank[15] and Sim and Pattle.[25] Melville measured airway resistance in terms of specific airway conductance (sGaw) converting airway resistance and functional residual capacity to its reciprocal, thereby eliminating any changes in airway conductance or airway resistance due to changes in functional residual capacity.

Subjects were exposed to 1.5 to 10 ppm sulfur dioxide gas in a body plethysmograph for 1 to 60 minutes. Breathing by mouth was compared with breathing by nose. Sulfur dioxide at concentrations below 5 ppm caused significant decreases in sGaw, which varied with the route of inhalation: mouth inhalation caused a greater percentage decrease in sGaw than did nasal inhalation. At concentrations above 5 ppm for 1 minute or more, however, there was no significant difference between the decrease in sGaw with nose or mouth breathing. Thus, at this higher sulfur dioxide concentration, sufficient sulfur dioxide passed through the nasapharynx to stimulate tracheobronchial receptors. The decrease in sGaw (and thus increase in airway resistance) upon nasal breathing contradicts both Sim and Pattle's[25] findings that 5.1 and 30.8 ppm for 60 and 10 minutes, respectively, resulted in no changes in PFR upon nasal breathing and Frank's[15] observation that exposures to sulfur dioxide at 15 and 29 ppm produced increases in PFR for 75 percent of subjects using mouth breathing and only 25 percent of nose-breathing subjects. Melville's findings supported Frank's[15] report that 4 to 7 ppm of sulfur dioxide increased airway resistance within the first minute with no further increases after 5 minutes, i.e., rapid acclimation to the mechanical pulmonary effects occurred.

Anderson et al.[22] measured a series of variables indicative of airway resistance over a 3-day period. Subjects breathing by nose were first exposed to sulfur dioxide over a 1- to 1.5-hour period to allow gradual tolerance to irritant effects. They were then subjected to sulfur dioxide at 1, 5, and 25 ppm with 1 day at each level for 7 to 8 hours each day for 3 consecutive days. At 1 ppm, the subjects experienced significant decreases in mid-tidal forced expiratory flow and nasal airway resistance, which further decreased with time. At 5 ppm, there were significant decreases in mid-tidal forced expiratory flow, nasal airway resistance, and nasal mucous flow rates, which also became more pronounced with time. At 25 ppm, the subjects experienced even greater significant decreases in mid-tidal forced expiratory flow, nasal airway resistance, nasal mucous flow rates, and forced expiratory volume

in 1 second. There was no change in the closing volume for any of the three trials. It is significant that sulfur dioxide concentrations as low as 1 and 5 ppm resulted in significant alterations in pulmonary functions.

Additional immediate respiratory effects in humans have been reported. Frank[15] exposed subjects to 4 to 16 ppm of sulfur dioxide gas by mouth for 30 minutes in a body plethysmograph. In addition to increases in pulmonary flow resistance, forced residual capacity and end-expiratory gas volume increased slightly but significantly, while no consistent changes were observed in pulmonary compliance, tidal volume, respiratory rate, or heart rate. Sim and Pattle[25] also reported no significant changes in the respiratory rate, heart rate, tidal volume, blood pressure, or maximum breathing capacity in subjects exposed to sulfur dioxide at 1.3 to 80 ppm for 10 to 60 minutes by mask or chamber (nasal breathing) for repeated, intermittent exposures. Drinker (cited in Lawther[96]) reported that inhalation of 1 to 8 ppm of the gas produced consistent changes in respiratory rate (shallower and more rapid breathing), increased heart rates, and decreased tidal volumes in human subjects. Frank et al.[19] reported that subjects exposed to sulfur dioxide at 1, 5, and 13 ppm alone and in combination with inert saline aerosol for 10 to 30 minutes by quiet mouth breathing in a plethysmograph exhibited a slight but significant increase in forced residual capacity at 13 ppm and a 7 percent decrease in maximum expiratory flow rate, but no significant changes in end-esophageal pressure, pulmonary compliance, total vital capacity, respiratory rate, heart rate, or tidal volume.

Lawther[78] randomly exposed 18 subjects (city and rural dwellers) to 0 to 20 ppm of sulfur dioxide gas for 10 minutes by nose and by mouth. No significant changes were observed in tidal volume, respiratory rate, or heart rate. These findings were confirmed by several investigators.[15,19,25] Amdur et al. (as cited in Lawther[96]) found that 1 to 8 ppm produced consistent changes in respiration and heart rates in 14 normal subjects. However, Lawther concluded that psychologic reactions (e.g., apprehension) may have played a role in Amdur et al.'s study and the mere detection of an unfamiliar smell could alter the respiratory pattern and heart rate.

In general, the immediate respiratory effects of short, high-level exposures to sulfur dioxide appear to be short-lived and reversible (with the exception of extremely intense exposures). Greenwald[4] noted that while evidence concerning chronic exposures is conflicting, occasional, even frequent, exposures to high concentrations of sulfur dioxide were not as harmful as continuous (working-day) exposures to lower, more tolerable concentrations. Frank,[15] Frank et al.,[19] and other investigators have reported transient increases in PFR of 72 percent above control values upon exposure to 13 ppm (10 to 16 ppm) of sulfur dioxide by quiet mouth breathing. It is not known whether further increases in PFR result from higher sulfur dioxide concentrations. It is also questionable whether a 72 percent increase in PFR is sufficient to impair the performance of individuals under stress (and as a result, having increased respiratory

rates and minute volumes). Since PFR levels decline quickly despite continued exposure and disappear upon termination of exposure, immediate irritant effects of sulfur dioxide may be of greater concern than immediate pulmonary effects in terms of combat efficiency and performance capabilities.

(2) Animal Experiments

Other data on the immediate pulmonary effects of exposure to sulfur dioxide are available in a number of animal studies. Dixon et al.[57] and Callanan et al.[58] exposed groups of New Zealand white rabbits, breathing through tracheal cannulas, to sulfur dioxide at 200 ppm for 2 and 5 minutes. Mean total lung resistance for both groups combined increased from a control value of 25.1 cm H_2O per liter per second to 67.5 cm. H_2O per liter per second. The bronchoconstriction was immediate but transient (occurring in the first minute of exposure) with the total lung resistance returning to 30.3 cm H_2O per liter per second soon after the termination of exposure. Mean inspiratory time approximately doubled while mean expiratory time decreased by about one-third. In addition, mean tidal volume increased by 4.3 mL and the Breuer-Hering reflex, which regulates lung inflation, was abolished in six of nine rabbits and was greatly reduced in the other three rabbits. Davies et al.[59] also confirmed that the Breuer-Hering reflex and pulmonary stretch receptors were abolished in rabbits by exposure to sulfur dioxide at 200 ppm, while the lung irritant receptors remained intact.

In a study by Balchum et al.,[52] tracheostomized dogs were exposed by cannula to sulfur dioxide at 1.8 to 148 ppm for 20 to 40 minutes. The nonelastic pulmonary resistance to breathing increased from 55 percent at 30 ppm to 212 percent at 21.5 ppm above control values for these dogs. Increases were observed within 9 seconds of sulfur dioxide exposure (in nine of ten dogs) and disappeared immediately following the end of exposure. Compliance of the lungs and thorax decreased by a mean of 6.2 percent within 10 to 15 minutes of gas inhalation and remained decreased at the end of the 30-minute postexposure period.

Alarie et al.[12] conducted a 78-week study of cynomolgus monkeys exposed to sulfur dioxide at 0.14, 0.64, 1.28, and 4.69 ppm. After 30 weeks at 4.69 ppm, one group of monkeys was accidently exposed to 200 to 1000 ppm of the gas for 60 minutes. After that point, the overexposed monkeys remained on filtered air for the rest of the study with pulmonary function tests performed once every 4 weeks. These monkeys had a wide variation in respiratory flow resistance on inspiration (unlike the other exposure groups), but the difference was not significant. Respiratory flow resistance on expiration, PFR, dynamic compliance work of inspiration, and work of expiration were also not significantly different from values from the other exposed groups or the controls. However, 3 weeks after the accident, the tidal volume of the overexposed group significantly increased, but this was not an immediate effect.

Frank and Speizer[60] reported that nasal flow resistance for 11 dogs exposed by nose to 7 to 16 ppm of sulfur dioxide for 10 or more minutes

increased with the gas concentration. For concentrations of 25 to 61 ppm, nasal flow resistance increased with the duration of exposure as well as the sulfur dioxide concentration. During the exposure period, there was no consistent change in direction of the resistance of lower airways (lung parenchyma and lower trachea); however, during the recovery period, the resistance of the lower airways significantly increased compared to control values. Flow resistance of the larynx also rose as often as it fell during exposure and recovery phases. For all sulfur dioxide levels, compliance was slightly but significantly lower during exposure than during either control or recovery periods (15 to 20 minutes after exposure). Minute ventilation, tidal volume, and respiratory rate also decreased significantly. No consistent trends were observed for end-expiratory volume, end-expiratory transpulmonary pressure, or lung volume. In a second series of experiments, dogs were exposed by tracheal cannulas to sulfur dioxide at 60 to 230 ppm delivered either to the lower airways (lower trachea and lung) or to the tracheolaryngeal segment for 10 to 15 minutes. Exposure of the lower airways to sulfur dioxide resulted in findings qualitatively similar to findings when the tracheolaryngeal segment was exposed: a tendency toward bronchoconstriction was greatest during the first few minutes of exposure, and soon tapered off. Direct exposure of the isolated tracheolaryngeal segment increased resistance from 59 to 157 percent above control values. Lower airway exposure produced resistance changes ranging from -20 percent to +40 percent. However, the magnitude of this change in resistance was clearly greater when the lungs were exposed directly (mean increase of 172 percent above controls). All changes were quickly reversible except for lower airway flow resistance after exposure by nose.

Lee and Danner[61] exposed guinea pigs to sulfur dioxide at 6 to 310 ppm for 60 minutes. Most of the animals exposed to more than 18 ppm experienced increases in tidal volumes and decreases in respiratory rates, whereas animals exposed to 6 to 18 ppm of the gas for 30 minutes exhibited tidal volume decreases ranging up to 38 percent, with poorly defined increases in respiratory rates. During the 60-minute recovery period, respiratory rate and tidal volume returned to control values in animals that had been exposed to less than 180 ppm of sulfur dioxide. At 300 to 310 ppm, recovery was still incomplete at 60 minutes. At lower concentrations, maximum increases in tidal volumes occurred within 15 minutes, but at these higher concentrations, tidal volumes continued to increase for 30 minutes (i.e., the exposure duration).

Kahana and Aronovitch[62] exposed rats to sulfur dioxide at 805 and 1226 ppm for 2 and 3 hours, respectively. Mean body weight increased insignificantly above controls for both exposure groups. For rats exposed to 805 ppm, maximal alveolar surface tension and minimal surface tension both decreased significantly ($p < 0.001$ and $p < 0.025$, respectively) compared to controls. Lung weight, hysteresis area, and the extract stability index increased insignificantly compared to control values.

For animals exposed to 1226 ppm, significant changes included increases in lung weight ($p < 0.005$) and the extract stability index ($p < 0.025$) and decreases in maximal surface tension ($p < 0.001$) and minimal

surface tension ($p < 0.005$). The hysteresis area increased insignificantly from control values. Histopathologic examination of the rats exposed to 805 ppm revealed no gross changes, whereas in the group exposed to 1226 ppm, there were changes suggestive of pulmonary edema, hyperemia, and focal atelectasis. Some animals experienced severe, persistent dyspnea and foamy tracheal secretions, but most animals experienced only transient respiratory difficulties. The findings indicated a sulfur dioxide-induced increase in pulmonary surfactant activity, possibly as an adaptive mechanism for the prevention of pulmonary edema. It is not known whether the surfactant changes observed in this study were reversible since no recovery periods were incorporated into the study.

In general, the immediate respiratory effects of exposure to sulfur dioxide observed in different animal species varied with the gas concentration and duration of exposure. Pulmonary effects observed in several species (rabbits, guinea pigs, cats, and dogs) qualitatively resembled the effects observed in humans. However, the concentrations and times necessary to produce these effects varied widely. The results of animal experiments, especially those in which nonphysiologic conditions were arranged, should be extrapolated to humans only with great caution. The two long-term experiments[12,63] provide evidence that moderately low- and high-level, long-term exposures may produce pulmonary damage. The case of the monkeys[12] accidentally exposed to between 200 and 1000 ppm of sulfur dioxide for 1 hour after 30 weeks at 4.69 ppm of the gas suggested that short, extremely intense gas levels may induce delayed respiratory damage in humans. From the military standpoint, however, it is unlikely that in training or combat a soldier would be exposed to such high concentrations. Further, the irritant effects at these concentrations might be intolerable to the point that personnel would evacuate the exposure site if at all possible.

b. Mechanism of Bronchoconstriction

The mechanism of bronchoconstriction has been investigated in two studies using human subjects or animals.

(1) Human Study

In an attempt to investigate the mechanism of bronchoconstriction in response to sulfur dioxide exposure, Nadel et al.[48] exposed subjects to sulfur dioxide at 4 to 6 ppm by mouth breathing for 10 minutes in a body plethysmograph. The mean ratio of airway resistance to thoracic gas volume decreased from control values by 39 percent with the time of onset ranging from 10 seconds to 4 minutes. The airway conductance usually decreased maximally within the first minute of exposure, leveling off thereafter. Other subjects were then subcutaneously injected with atropine (1.2 to 1.8 mg) 20 minutes before exposure. No significant changes from controls were observed in the airway conductance to thoracic gas volume ratio.

(2) Animal Studies

Nadel et al.[48] performed pulmonary resistance experiments on tracheostomized cats with sulfur dioxide delivered to their isolated upper or lower airways. Administration of the sulfur dioxide resulted in similar increases in lung resistance whether delivered to the upper or lower airways; administration of sulfur dioxide in combination with atropine resulted in no significant changes in lung resistance from controls, whether delivered to the upper or lower airways. With cooling of the vagosympathetic nerves, sulfur dioxide administration did not increase lung resistance above control levels; subsequent rewarming of the nerves resulted in the usual increases. These results were observed whether sulfur dioxide was delivered to the upper or lower airways. This suggested that the bronchoconstrictive reflex was mediated by the vagus nerve, which controls the smooth muscle tone of the airways, and that the nerve receptors are located in both the upper and lower airways. All effects were reversible.

Salem and Aviado[64] exposed dogs to sulfur dioxide at 200 to 850 ppm for 1 to 4 minutes through a tracheal cannula. In most dogs, initial bronchodilation was followed by bronchoconstriction. Atropine sulfate administration resulted in two cases of bronchodilation and two cases of a biphasic response (bronchodilation followed by bronchoconstriction). The explanation for this phenomenon remained uncertain, suggesting, however, that in addition to vagal stimulation other mechanisms might be involved, such as local bronchodilation caused by sulfurous acid or the action of histamine on smooth muscle. However, it should also be noted that their use of morphine and chloralose as anesthetics may have altered the bronchial response.

d. Interactions of Sulfur Dioxide with Other Compounds

(1) Effect of Ammonia

Some data suggest that the presence of ammonia results in a diminution of the effects of sulfur dioxide exposure. In a study by Sim and Pattle,[25] subjects were exposed to sulfur dioxide at 9.9 ppm for 60 minutes in a chamber with ammonia gas (at unspecified concentration) introduced into the chamber after 50 minutes of exposure. These subjects experienced a cessation of the irritant and bronchoconstrictive effects upon the addition of ammonia.

Kosmider and Ludyga[27] reported that guinea pigs exposed to ammonia in combination with sulfur dioxide had less severe disturbances in acid-base equilibrium and enzymatic activities in blood and tissue than did animals exposed to the same concentration of sulfur dioxide alone.

Jonek et al.[26] reported that rats exposed to ammonia with sulfur dioxide ($^{35}SO_2$) had lower concentrations of ^{35}S in serum and various organs than did rats exposed to sulfur dioxide alone.

These reported effects are attributed to an interaction of sulfur dioxide and ammonia, which results in the formation of ammonium sulfate.

(2) Sulfur Dioxide in Combination with Aerosols

Studies using both human and animal subjects have been conducted to determine if exposure to sulfur dioxide in combination with aerosols has any effect on the level of pulmonary changes observed in response to exposure to sulfur dioxide alone.

(a) Human Studies

In a study by Frank,[15] subjects were exposed to sulfur dioxide at each of three concentration ranges (1 to 2 ppm, 4 to 7 ppm, and 14 to 17 ppm) alone and then in combination with sodium chloride aerosol (average concentration: 18 mg per m^3; range: 10 to 30 mg per m^3; particle size: 0.04 to 0.15 μm) for 30 minutes by mouth. The sulfur dioxide-aerosol mixture produced changes in PFR similar to those observed with the gas alone. However, in these subjects as well as in other subjects in which the sequence of exposure was reversed, it was always the initial exposure (whether to the gas alone or the gas-aerosol combination) that evoked the greater increases in PFR.

Snell and Luchsinger[65] exposed human subjects by mouthpiece to each of several mixtures including distilled water aerosol; saline aerosol (6.0 to 8.0 m); sulfur dioxide alone at 0.5, 1.0, and 5 ppm; sulfur dioxide at 0.5, 1.0, and 5 ppm in combination with saline aerosol; and sulfur dioxide at 0.5, 1.0, and 5 ppm in combination with distilled water aerosol. Measurements were taken by spirometry upon forced expiration at maximum effort. Exposure to sulfur dioxide at 0.5 ppm or in combination with saline aerosol alone produced no significant changes in the maximum expiratory flow rate at one-half vital capacity (MEF_{50} percent VC). Exposures to 1 ppm and 5 ppm of sulfur dioxide alone produced significant decreases in MEF_{50} percent VC, with a decline in flow rates as gas concentration increased (suggesting a dose-response relationship). Combinations of sulfur dioxide and inert saline aerosol produced a significant decrease in MEF_{50} percent VC only for 5 ppm of sulfur dioxide, with no indication of a synergistic response. Combinations of distilled water aerosol with all levels of sulfur dioxide (0.5, 1 and 5 ppm) produced significant decreases in MEF_{50} percent VC. Unlike sulfur dioxide alone, however, the decreases in flow rates for all three levels of the gas aerosol were essentially identical, with no indication of either synergism or a dose-response relationship. The differences in response between saline-gas and distilled water-gas aerosols were attributed to evaporative losses and the greater particle size for the saline (6 to 8 μm) compared with the distilled water (0.3 μm).

Burton et al.[66] exposed 10 subjects to approximately 2 ppm of sulfur dioxide gas alone and in combination with sodium chloride aerosol (mean: 2.2 mg per m^3, 0.25 μm diameter) for 30 minutes. Measurements obtained by spirometry and plethysmography during quiet mouth breathing and hyperventilation revealed no significant changes in PFR, airway resistance, lung compliance, airway conductance, specific airway resistance, or thoracic gas volume.

Frank et al.[67] exposed subjects orally to sulfur dioxide at 1 to 17 ppm in combination with sodium chloride aerosols. This study also indicated that with repeated exposure to the gas alone or the gas-aerosol mixture (in either order), the first exposure produced the greater increase in airway resistance. Subjects were exposed by nose for 15 minutes to 5 ppm of sulfur dioxide alone, 0.5 ppm of the gas in combination with distilled water aerosol, and 5 ppm of the gas combined with distilled water aerosol. These results were then compared with those of the previous trial (mouth breathing). For the 0.5 ppm and 5 ppm sulfur dioxide-aerosol mixtures, maximum expiratory flow rates decreased less with nasal breathing than with mouth breathing, but the differences were not significant. For the two sulfur dioxide-distilled water aerosol mixtures, mouth inhalation produced conductance decreases of 20 percent compared to controls, while nasal inhalation produced conductance increases (with the 5-ppm sulfur dioxide-aerosol, conductance significantly decreased for both mouth and nasal inhalation, proportional to the gas concentration). For the sulfur dioxide alone (5 ppm), conductance decreased approximately 25 percent for both mouth and nasal breathing. Conductance changes were present at 4 minutes of inhalation, increasing only slightly after 12 minutes of exposure. Frank et al.[19] reported similar results for PFR, with the exception of exposures at 1 ppm of sulfur dioxide.

The differences between some of these studies may be explained by the methods of measurement: Snell and Luchsinger[65] measured airflow by spirometry with subjects forcefully expiring at maximum effort, while Frank et al.[19] employed a body plethysmograph, with measurements taken during quiet breathing and averaging flow resistance for both inspiration and expiration in the mid-tidal range of lung volume. The difference in responses between saline and distilled water aerosol may be explained by the large particle size of the saline aerosol. Aerosols of greater particle sizes tend to produce lesser effects in the lower respiratory tract due to their greater likelihood of deposition in the upper respiratory tract.[65] The reason for the significant increase in conductance at 5 ppm of sulfur dioxide-aerosol mixture by nasal inhalation compared to mouth inhalation is unclear. It was postulated that local vasoconstriction due to the acidity of the gas-aerosol mixture decreased nasal surface area available for absorption, thereby allowing greater passage of the mixture into the lower airway. In summary, however, these studies provide no clear evidence that administration of aerosols would potentiate the toxic effects of sulfur dioxide exposure.

(b) Animal Studies

Amdur[68] exposed guinea pigs to sulfur dioxide at 89 ppm alone or in combination with sulfuric acid mist at 2 ppm for 8 hours. Data for exposures to sulfuric acid mist at 2 ppm alone, however, were taken from previous experiments. The combination of the gas and mist resulted in the greatest decreases in growth (expressed as body weight), extensive lung pathology, severe respiratory symptoms, and intense irritant symptoms. Neither substance alone produced such marked results nor such prolonged recovery periods. Re-exposure to the gas-mist mixture resulted in irreversible growth impairment, widespread hemorrhage, and consolidation of the lungs, and, consequently, markedly aggravated respiratory distress.

The changes induced by the gas-mist combination were clearly more extensive than those produced by sulfur dioxide or sulfuric acid mist alone.

Corn et al.[42] exposed cats by nose, endotracheal catheter, and tracheal cannula to sulfur dioxide at 15 to 25 ppm, sodium chloride aerosol at 10 mg per m^3, and the gas-aerosol combination for up to 30 minutes. The majority of tracheostomized animals showed no pulmonary function changes in response to administration of sulfur dioxide at 20 ppm alone or in combination with sodium chloride aerosol, indicating an absence of synergism. Animals breathing by endotracheal catheter or face mask (nose) showed significant increases in pulmonary flow resistance, which were reversible after exposure ceased. The lesser responses evolved by inhalation by tracheal cannula suggested that nasopharyngeal receptors proximal to the tracheal cannula were bypassed.

McJilton et al.[69] exposed guinea pigs to sulfur dioxide at 1.1 ppm alone and in combination with sodium chloride aerosol, at 900 to 1000 µg per m^3, or water vapor for 1 hour at diffent relative humidities. Lung resistance increased significantly above control levels only for those animals exposed to the sulfur dioxide-sodium chloride aerosol-water vapor mixture at high relative humidities (i.e., greater than 80 percent) with lung resistance further increasing with time. Findings indicated a synergistic response between the mixture and a high relative humidity. McJilton et al.,[70] in a similar experiment, repeatedly exposed guinea pigs to sulfur dioxide at 1 ppm, sodium chloride aerosol at 1 mg per m^3, and the gas-aerosol in combination at low (less than 40 percent) and high (greater than 80 percent) relative humidities for 60 minutes with 60-minute recovery intervals. Again, lung resistance increased only with exposure to the gas-aerosol mixture at high relative humidities, with a mean increase of 47 percent above control levels. The increase in lung resistance took place in the first 15 minutes of exposure, and was sustained for the exposure duration. Dynamic lung compliance significantly decreased during the same exposure. Administration of the gas-aerosol mixture at high relative humidity was thought by the authors to produce a synergistic response. This effect was ascribed to absorption of the highly water-soluble sulfur dioxide into the aerosol droplet before inhalation, thus decreasing the gaseous sulfur dioxide concentration to as low as 60 percent of the administered level and resulting in decreased penetration of the lungs by sulfur dioxide gas.

As with human studies, animal studies do not present clear evidence of a synergistic effect when saline aerosols are administered with sulfur dioxide.

(3) Sulfur Dioxide with Benzo(a)pyrene

Laskin et al.[43] exposed rats to sulfur dioxide alone at 10 ppm for 6 hours per day, 5 days per week and sulfur dioxide at 3.5 ppm in combination with benzo(a)pyrene at 10 mg per m^3 for 1 hour per day, 5 days per week, both for more than 2 years. Survival was exceptionally high with significant mortality occurring only after 80 weeks of exposure. However,

of 21 rats exposed to the sulfur dioxide-benzo(a)pyrene combination, two rats developed squamous cell carcinoma of the lung by 655 and 698 days, with renal metastasis, a third rat showed advanced squamous cell metaplasia at 623 day and five rats were found to have squamous cell carcinoma of the lung at 547, 603, 655, and 794 days. Two additional rats exhibited advanced squamous cell metaplasia at 485 and 715 days. Neither sulfur dioxide alone nor in combination with benzo(a)pyrene produced bronchogenic carcinomas. The results suggested a promoting effect for sulfur dioxide similar to the effects observed by Peacock and Spence.[71]

F. ACCLIMATION AND TOLERANCE

A number of animal and human studies document the existence of immediate tolerance and gradual acclimation (usually during industrial exposure) to the irritant, respiratory, and lethal effects of sulfur dioxide exposure.

1. Acclimation

Acclimation is the physiologic adjustment of an individual to environmental changes, whereby various offensive environmental components (in this case sulfur dioxide) become less objectionable. Since this process can require months or even years to occur, most data are derived from epidemiologic studies of sulfur dioxide-exposed workers.

Kehoe et al.[24] reported the acclimation or the physiologic adjustment of new workers to the irritant and respiratory effects of repeated, intermittent long-term exposures to sulfur dioxide reported as averaging 20 to 30 ppm but ranging from 5 to 70 ppm. This phenomenon was observed to occur in 80 percent of the workers. Individual variation in adaptation was extremely great, with most workers adapting rather quickly.

This study reported a variety of adverse health effects, significantly correlating with the frequency of severe exposures. Following the onset of acclimation, the workers were able to perform tasks without the appearance of the initial irritant and respiratory symptoms. However, acclimated workers abruptly exposed to sulfur dioxide at more intense concentrations experienced greatly intensified initial irritant and respiratory symptoms.[4,24]

Anderson[72] reported acclimation among workers exposed daily to sulfur dioxide at up to 25 ppm. Again, there was wide individual variation for the time required for adaptation, but most workers felt that they were almost totally adapted within the first year. No adverse health effects were reported in workers exposed up to 19 years.

Humperdinck (cited in Greenwald[4]) observed that workers exposed daily for more than 1 year to 23 to 105 ppm of sulfur dioxide demonstrated abnormal lung sounds on chest auscultation (confirmed by X-ray as chronic bronchitis and emphysema).

Melville[28] and others[4,41,46] reported that despite the acclimation to the effects of sulfur dioxide, the absence of irritant symptoms should not be accepted as evidence that no harmful effects occur, since prolonged exposure may eventually compromise pulmonary function.

Acclimation is attributed by some authors[19,28] to a gradual depression of tracheobronchial nerve reflexes and by others[52] to a direct action of sulfur dioxide on bronchial smooth muscle.

2. Tolerance

Tolerance refers to the rapid adaptation that begins to occur immediately or within minutes after initial exposure to sulfur dioxide. It results in a diminished response to the irritant effects of the gas and, in some cases, changes in pulmonary function are also diminished.

a. Human Experiments

At low to moderate concentrations of sulfur dioxide (1 to 25 ppm), tolerance or rapid adaptation to the irritant symptoms and mechanical pulmonary effects has been observed within a few minutes of exposure, whether subjects breathed by mouth or by nose.[15,19,22,41] Frank[15] reported that humans exposed by mouth breathing to sulfur dioxide at 4 to 16 ppm for 10 to 30 minutes experienced the immediate onset of coughing, throat and upper chest irritation, and increased salivation, all of which subsided within the first few minutes of exposure. Pulmonary flow resistance increased within the first minute, reaching a maximum of 72 percent above control values within the first 5 to 10 minutes of exposure, partially decreasing thereafter but remaining above control levels throughout the exposure. The author explained the partial refractoriness of the pulmonary flow resistance as a vagosympathetic reflex adaptation, occurring whether the gas was inhaled by nose or mouth, continuously or discontinuously. Frank et al.[19] reported (in experiments similar to Frank's[15]) that irritant responses subsided within 5 minutes, while PFR peaked between 5 and 10 minutes of exposure, subsiding thereafter despite prolonged exposures to the gas. Chitty[41] reported that individuals exposed to 5 to 10 ppm of sulfur dioxide developed coughing, nasopharyngeal irritation, and bronchoconstriction, with the effects subsiding after several minutes.

Melville[28] reported significant decreases in specific airway conductance in subjects exposed by mouth or nose to sulfur dioxide at 2.5, 5, and 10 ppm for 10 minutes. The maximal decrease was reached in 5 minutes, with no further changes observed. Subjects exposed by mouth to 5 ppm of the gas for 1 to 60 minutes experienced significant decreases in specific airway conductance, peaking after 5 to 20 minutes with no further increases thereafter. Irritant symptoms of coughing, burning sensations

in the throat, and substernal pain were reported, some symptoms apparently continuing throughout the exposures and for up to a week after the experiment.

Nadel et al.[48] reported that mouth inhalation of 4 to 6 ppm of sulfur dioxide for 10 minutes significantly increased airway resistance in exposed subjects by a mean of 39 percent. Coughing and pharyngeal and substernal pain were experienced initially but subsided during the exposure. In most cases, airway conductance decreased maximally within the first minute of exposure, with no further decreases.

Lehmann (cited by Greenwald[4]) observed that workers chronically exposed to 6 to 30 ppm of sulfur dioxide appeared to be without discomfort. He and two associates (unaccustomed to the gas), when exposed to 6.5 and 11.5 ppm of sulfur dioxide for 10 to 15 minutes, experienced nasal irritation, which quickly subsided. However, with exposure to 30 to 57 ppm of sulfur dioxide, rapid adaptation did not occur; the irritant and respiratory symptoms remained extremely disagreeable and exposures never voluntarily exceeded 15 minutes.

Anderson et al.[22] reported that human subjects, even those acclimated to sulfur dioxide through occupational exposure, are more sensitive to the irritant effects of sudden or abrupt increases in sulfur dioxide concentration than to those same increases made through gradual increments in concentration. Human subjects exposed to sulfur dioxide at gradually increasing concentrations tolerated even 25 ppm very well and extreme discomfort was never recorded. Experimenters who occasionally had to enter the chamber experienced irritation at those same concentrations; 5 ppm caused strong discomfort and coughing, 25 ppm initially caused almost intolerable discomfort, which gradually eased with time.

b. Animal Experiments

Evidence of tolerance to short high-level exposures of sulfur dioxide is available in a few recent animal studies. However, the extrapolation of quantitative information between species (or even within a species) is hazardous.[41] Ronzani (cited by Greenwald[4]) exposed rabbits, guinea pigs, and pigeons to 50 and 500 ppm of sulfur dioxide for 6 to 7 hours per day for 1 month. Animals exposed to 500 ppm immediately experienced restlessness, sneezing, and lacrimation, with symptoms subsiding by the end of the second exposure week. Animals exposed to 50 ppm of the gas showed no obvious ill effects. Goldring et al.[73] exposed hamsters to 650 ppm of sulfur dioxide for 3 hours per day, 5 days per week for a total of 636 exposure hours. On initial exposure, the animals exhibited restlessness, rubbing of the nose, and dyspnea. Although the severity of these symptoms declined after 1 week, acclimation to the irritant and pulmonary effects did not fully develop.

Asmundsson et al.[13] reported the differential effects of abrupt and gradual exposures to high levels of sulfur dioxide. Hamsters abruptly exposed to 400 ppm of sulfur dioxide for 5 hours per day immediately developed respiratory distress, with all animals dying between 45 minutes and 6.3 hours of exposure. Asmundsson et al. then gradually exposed hamsters to 400 ppm of sulfur dioxide by increasing concentrations from 250 to 400 ppm (over 4 days) for 5 hours per day, 5 days per week for a total of 35 exposure hours. No deaths were reported for this group in spite of the development of respiratory tract damage, which was revealed at necropsy. These and other findings[4,22,28,41] indicate that the development of tolerance to sulfur dioxide should not be accepted as evidence that no harmful effects occur from repeated, nonlethal exposures.

3. Significance of Acclimation and Tolerance Phenomena

In humans, the development of rapid adaptation to the irritant and respiratory symptoms of moderately high concentrations of sulfur dioxide, such as 25 ppm, is of interest from the military standpoint. The rapid tolerance or adaptation that occurs would cause a subsidence of the irritant and pulmonary symptoms after several minutes of exposure. However, the tolerance resulting from gradual increments in the sulfur dioxide concentration that might reduce or eliminate the irritant symptoms entirely would not be expected in combat situations where the rises in sulfur dioxide concentration may be abrupt. Also, rapid development of tolerance has not been demonstrated in humans for sulfur dioxide at concentrations exceeding 25 ppm.

In addition, Kehoe et al.[24] and Lehmann (cited by Greenwald[4]) have demonstrated that even in rapidly adapted individuals and workers acclimated over a period of years to repeated daily exposures at concentrations of 20 to 30 ppm, abrupt exposures to higher concentrations resulted in intensified symptoms similar to those experienced on initial exposure to the gas. Kehoe et al.[24] reported that abrupt, heavy exposures of acclimated workers caused coughing, sneezing, conjunctival irritation, soreness of the throat and larynx, thirst, weakness, both transitory and prolonged chest pains, loss of appetite, and polyuria. Lehmann[4] observed that individuals who rapidly adapted to 11.5 ppm of sulfur dioxide (after initially undergoing intense nasal irritation) experienced such extremely disagreeable symptoms upon abrupt exposure to 30 to 57 ppm that none could endure the exposure for longer than 30 minutes. At this abruptly increased concentration of the gas, rapid tolerance did not occur.

For the soldier in training or combat, the implications are that rapid adaptation or tolerance may occur, but further abrupt increases of sulfur dioxide concentration will negate the adaptive response and result in a recurrence of the irritant symptoms; and if the gas concentration exceeds 30 ppm, the soldier may not adapt to the gas but may instead experience intolerable symptoms.

G. GENERAL CONSIDERATIONS UNDERLYING THE TOXICITY OF SULFUR DIOXIDE

Dalhamn and Sjoholm[74] considered the major factors determining the immediate toxicity of a pulmonary irritant gas to be its absorptiveness in the upper respiratory passages and its ability to impair ciliary activity and thus inhibit clearance. An irritant gas that impairs ciliary activity at low concentrations and is absorbed to only a slight degree in the upper respiratory passages would prove to be more toxic than a gas with the reverse characteristics. Based on these criteria, sulfur dioxide is moderately toxic since it has a high rate of absorption in the upper respiratory tract, but, relative to other toxic gases, the threshold of sulfur dioxide required to effect ciliary stasis is low.

1. Effect on Ciliary Activity

a. In Vitro Experiments

A few in vitro studies have been conducted to determine the effect of sulfur dioxide on the ciliary activity of the pulmonary tract. In rabbit trachea exposed in vitro to sulfur dioxide at 20 to 30 ppm for 5 minutes, ciliary activity was arrested, whereas exposures to 500 to 1000 ppm of ammonia and 150 to 200 ppm of nitrogen dioxide were required to elicit the same effects.[74] Cralley (as cited in Dalhamn and Strandberg[75]) reported ciliary stasis in rabbit trachea exposed in vitro to sulfur dioxide at 50 ppm for 1 minute and to 30 ppm for 5 minutes. Strandberg reported ciliary stasis in rat trachea exposed in vitro to 12 ppm for 5 minutes and to 50 ppm for 1 minute.[76]

b. Animal Experiments

Studies using live animals, however, have produced markedly different results, with much higher sulfur dioxide levels required to inhibit ciliary activity. The higher levels are presumably required to compensate for the high rate of sulfur dioxide absorption in the nasopharyngeal mucosa of the live animal. Dalhamn and Strandberg[75] exposed live rabbits by nose to sulfur dioxide at 100, 200, and 300 ppm for up to 45 minutes. At 100 ppm, ciliary activity was not affected; at 200 ppm, ciliary activity decreased with time; at 300 ppm, ciliary stasis was practically complete at 45 minutes.

Because of the ability of sulfur dioxide to inhibit ciliary activity, the inspired toxic gas will remain in the lungs for a longer period of time and thus, presumably, cause more localized damage. Clearance of other foreign materials is also inhibited when ciliary activity is impaired, but these effects are usually somewhat delayed.

2. Effect on Mucociliary Clearance

Inhibition of the clearance phenomenon, of which ciliary activity is an integral part, can have long range or delayed health implications since other environmental pollutants, including gases and particulate matter as well as infective microorganisms, will tend to accumulate in the lungs to

a greater extent than if clearance were normal. The logical consequence of such an inhibition would be an increased incidence, severity, and/or duration of respiratory tract infections.

a. Occupational Exposure Data

Kehoe et al.[24] reported that workers occupationally exposed to sulfur dioxide had an increase in the duration but not the incidence of colds. Data of this nature suggest, at least indirectly, that sulfur dioxide exposure has a negative effect on clearance; but, more importantly, they indicate that a potential for delayed health effects exists for the exposed individual.

b. Human Experiments

Anderson et al.[22] exposed subjects by nose to sulfur dioxide for 1 day at each of three levels (1, 5, and 25 ppm) for 6 hours each day. Significant decreases in nasal mucus flow rates and nasal clearance were observed at 5 and 25 ppm; these effects were greatest in the anterior part of the nose.

Newhouse et al.[77] exposed exercising (mouth-breathing) subjects to radiolabeled technetium 99m albumin-saline aerosol, then to sulfur dioxide at 5 ppm, sulfuric acid at 1 mg/m^3, and sulfur dioxide-sulfuric acid in combination at those same concentrations for up to 2.5 hours by mouth. All three exposures resulted in increased tracheobronchial clearance, with the combination producing the greatest increase.

Wolff et al.[20] exposed subjects to a radiolabeled technetium 99m albumin-saline aerosol and to sulfur dioxide at 5 ppm for up to 3 hours by mouth. Results indicated no impairment of tracheobronchial clearance compared to control values.

The results of these studies are obviously not conducive to any generalizations about the effects of sulfur dioxide exposure on tracheobronchial clearance in humans. Discrepancies in results cannot be resolved by differences in oral versus nasal inhalation of sulfur dioxide as shown by the Anderson et al.[22] and Wolff et al.[20] studies.

c. Animal Experiments

Spiegelman et al.[78] repeatedly exposed three miniature donkeys to monodisperse ferric oxide-technetium 99m particles and sulfur dioxide at 27 to 713 ppm by nose. No change in tracheobronchial clearance was observed at gas concentrations below 300 ppm. Above 300 ppm, tracheobronchial clearance decreased, a maximum effect being observed with exposures from 557 to 713 ppm, which were characterized by a sixfold increase in mean bronchial clearance time.

Ferin and Leach[79] exposed rats to multiple, intermittent doses of sulfur dioxide at 0.1, 1, and 20 ppm for 7 hours per day, 5 days per week for a total of 70 to 170 hours and 10 to 25 days. The animals were then

exposed to titanium dioxide (TiO_2) particles. Rats exposed to 0.1 ppm of the gas for up to 23 days had lung clearance values equivalent to or greater than control values. At 1 ppm of the gas, lung clearance had an inverse relationship with the number of exposures. With 10 to 20 days of exposure at 1 ppm, clearance was at control levels or better; with 25 days, lung clearance was significantly decreased ($p < 0.01$), but the effect was reversible. With exposure to sulfur dioxide at 20 ppm for 11 days, lung clearance was significantly decreased ($p < 0.01$) compared to control values. From their data, the authors calculated that clearance of TiO_2 particles was inversely proportional to sulfur dioxide concentration multiplied by the duration of exposure to the fourth power:

$$\text{clearance} \propto \frac{1}{C \times t^4}$$

Short-term higher concentrations resulted in less inhibition of clearance than long-term low concentrations. All observed effects were reversible.

Knauss et al.[80] repeatedly and intermittently exposed rats to sulfur dioxide at 600 to 700 ppm for 3 hours per day for 9, 18, and 30 total exposure hours. Tracheal mucus clearance decreased two- to four-fold as a function of exposure time. Retention of cellular material and mucus was increased with goblet cells also increasing in number after 30 hours of gas exposure.

Reid[81] exposed rats to sulfur dioxide at 300 to 400 ppm for 5 hours a day, 5 days per week for 6 weeks. Observation of pulmonary tissue of animals sacrificed at weekly intervals revealed that goblet cells were distended and increased in number, both in large bronchi and in peripheral bronchioli, which normally lack goblet cells. Excessive mucus was observed in the bronchial lumen. The increased number of goblet cells persisted for at least 3 months after the cessation of exposure. There was evidence of an extremely slow return to normal; several animals exhibited a regression in the number of goblet cells with increasing post-exposure time. The results of exposure resembled the course of chronic bronchitis in humans: an increase in the number of goblet cells, excess secretion of mucus, and the formation of new goblet cells in narrow peripheral airways where they are usually absent.

Gause and Barker[82] exposed rats to sulfur dioxide at 5 and 20 ppm for 24 hours per day for up to 7 days. Rats were sacrificed and examined after 0.5, 1, 2, and 5 hours and 7 days of exposure, and at postexposure times of 96, 144, and 192 hours. Nasal mucus samples were electrophoresed to separate mucus glycoproteins. Samples from rats exposed to 5 ppm of the gas showed at least two discrete new bands and samples from rats exposed to 20 ppm showed at least five new bands, both sets in the acidic portion of the mucus glycoprotein. The new bands appeared to be the result of cross-linking among glycoprotein, this polymerization resulting in the formation of nonfunctional mucus glycoprotein and, consequently, poor mucus transport. These aggregates were not cleared by normal physiologic processes, but persisted for over a week postexposure for both the 5 and 20 ppm sulfur dioxide exposures. This formation of

large aggregates of cross-linked glycoprotein induced by sulfur dioxide inhalation could explain the observed decreases in nasal mucus flow rates observed in humans and the increased susceptibility to infection epidemiologically related to sulfur dioxide exposure.

Barry et al.[83] intermittently exposed rats to sulfur dioxide at 300 ppm for 6 hours per day for 10 consecutive days. Hypersecretion of mucus and a consequent increase in the amount of mucus reaching the alveoli were observed. Activity was increased in four lysosomal hydrolytic enzymes (i.e., β-glucuronidase, β-galactosidase, N-acetyl-β-glucosamidase, and acid phosphatase) in alveolar macrophages, in the peribronchiolar region of the lung, with a marked increase of acid phosphatase activity in free alveolar cells throughout in the lung parenchyma. Acid phosphatase is thought to be involved in the digestion of foreign material engulfed by phagocytosis. The increase in enzyme activity in the alveolar macrophages appeared to be a direct response to sulfur dioxide-induced increases in mucopolysaccharide in the mucus.

Ferin and Leach[84] repeatedly and intermittently exposed rats to TiO_2 particles, then to sulfur dioxide at 0.1 to 20 ppm for 7 hours per day, 5 days per week for a total of 10 to 25 exposures. Sulfur dioxide at 1 ppm for 25 exposures and at 20 ppm for 11 exposures decreased ($p < 0.01$) tracheobronchial clearance by 30 percent. However, clearance increased by 28 percent above control levels for sulfur dioxide at 0.15 ppm for 10 exposures, 0.1 ppm for 23 exposures, and 1 ppm for 10 exposures. Therefore, no consistent effects of sulfur dioxide exposure on clearance could be derived from this study. All reported effects were reversible.

d. Comparisons of Human and Animal Data

It is difficult to compare the results of human and animal studies of tracheobronchial clearance. Human studies usually involved single exposures to low concentrations of sulfur dioxide; animal experiments usually involved single exposures to high concentrations or repeated intermittent exposures to either low or high concentrations. The results of part of the human study by Anderson et al.[22] that most closely resemble the results of animal studies indicated that with exposure to higher concentrations (25 ppm and above) for longer durations, some inhibition of tracheobronchial clearance may be observed.

3. Absorption

The results of various studies have shown that sulfur dioxide is rapidly absorbed by mucosal tissue, especially by the nasopharyngeal mucosa.

a. Human Experiments

Speizer and Frank[49] repeatedly exposed subjects by nose to sulfur dioxide at 16 ppm for 25 to 30 minutes. Approximately 99 percent of the inspired gas was nasally absorbed. However, in spite of this high absorptive rate, subjects reported coughing and slight irritation of the

posterior pharynx, which indicated that sufficient sulfur dioxide had reached the larynx to initiate changes in bronchomotor tone and irritant responses.

Frank[15] exposed subjects by nose and mouth to sulfur dioxide at 15 and 29 ppm for 25 minutes. Pulmonary flow resistance increased significantly in 75 percent of subjects breathing by mouth but only in 25 percent of the subjects breathing by nose, suggesting a lesser degree of lower airway penetration by sulfur dioxide in nose-breathing subjects. In addition, mouth-breathing subjects experienced coughing and chest irritation, while nose-breathing subjects experienced only infrequent nasal irritation. Further, subjects exposed by nose to sulfur dioxide at 12 ppm for 30 minutes absorbed approximately 99 percent of the inhaled gas in the naso- pharyngeal mucosa. Desorption from the mucosa at expiration resulted in a net uptake by the airways of 84 percent of inspired sulfur dioxide.

b. Animal Experiments

Frank et al.[30] exposed the surgically isolated upper airways of dogs to 1 and 10 ppm of sulfur dioxide by mouth and nose for up to 5 minutes. Nasal absorption of the gas was 99 percent at 1 and 10 ppm for 5-minute exposures at flow rates of 3.5 and 35 liters per minute. Mouth absorption of the gas was approximately 99 percent at 1 and 10 ppm for mean exposure times of 4.6 and 3.2 minutes at a flow rate of 3.5 liters per minute. However, mouth absorption of the sulfur dioxide fell to 34 percent when 1 ppm was delivered at a flow rate of 35 liters per minute for a mean exposure time of 1.6 minutes. These results indicated that the nasal airways have a greater absorption potential than does the mouth; thus, the mode of breathing will strongly influence the proportion of inspired sulfur dioxide that will reach the lower airways. The rate of gas flow is also an important factor in this process since high flow rates can exceed the absorptive capacity of the oral mucosa.

Similarly, Dalhamn and Strandberg[75] reported that upper respiratory tract absorption of sulfur dioxide in spontaneously breathing rabbits averaged 95 to 99 percent at gas concentrations up to 308 ppm. In contrast, sulfur dioxide forcibly passed through rabbit nasal airways was more poorly absorbed at concentrations exceeding 200 ppm, with only 85 percent absorption at 318 ppm. Sulfur dioxide inhaled through the mouth at 175 to 213 ppm for 45 minutes had a mean absorption rate of less than 85 percent, decreasing with time. Rabbits exposed to sulfur dioxide at 400 and 6000 ppm by nose or tracheal cannula had absorption rates of 46 percent regardless of the mode of gas inhalation.

Strandberg,[76] however, in a study using rabbits exposed to sulfur dioxide by tracheal cannula, found that absorption rates in the lower respiratory tract were proportional to the gas concentration. Sulfur dioxide at high concentrations (up to 700 ppm) was absorbed by the lower airway at a rate of 95 to 98 percent, whereas at 0.1 to 0.5 ppm the absorption rate was 40 to 80 percent. These results were attributed to the greater degree of stimulation of the mucous glands by the higher sulfur dioxide levels, and thus an increased absorptive capacity. Frank et al.[51] nasally exposed the surgically isolated head and neck (i.e.,

nose, pharynx, larynx, and uppermost trachea) of dogs to radiolabeled sulfur dioxide at 22 ppm for 30 and 60 minutes. A minimum of 95 percent of the sulfur dioxide was absorbed by the upper airways. During the exposure, sulfur dioxide appeared in the expired air from the isolated lower airways, but at less than 1 percent of the exposure level. The gas was detectable in the expired lower airway gas for up to 60 minutes after exposure ended.

4. Distribution of Absorbed Sulfur Dioxide

Information on the blood levels and tissue distribution of sulfur dioxide and its metabolites is based solely on the results of animal experiments.

a. Blood and Urinary Distribution

Yokoyama et al.[85] exposed the isolated upper airways of dogs to radiolabeled sulfur dioxide ($^{35}SO_2$) at 22 and 50 ppm for 30 to 60 minutes. Blood ^{35}S rose progressively during exposure, decreasing only slightly after exposure ended. Plasma contained more ^{35}S than red blood cells. Two-thirds of the red blood cell ^{35}S was intracellular. More than half of the plasma ^{35}S was dialyzable, and the nondialyzable ^{35}S was bound to alpha-globulin proteins. The ratio of red blood cell ^{35}S to plasma ^{35}S was higher following in vivo exposure than in vitro exposure, suggesting an active transport uptake by red blood cells. Most of the urinary ^{35}S was excreted as inorganic sulfate (84 percent). ^{35}S was present in whole blood after 3 hours postexposure. Further, assuming all of the inhaled sulfur dioxide was nasally absorbed, then only 1 to 6 percent of the ^{35}S was excreted by the urine during the postexposure period at a rate proportional to ^{35}S in the blood and plasma.

Frank et al.[51] nasally exposed the isolated upper airways of dogs to radiolabeled sulfur dioxide ($^{35}SO_2$) at 22 ppm for 30 to 38 minutes and 60 minutes in a continuous unidirectional stream. More than 95 percent of the gas was absorbed in the upper airways and rapidly appeared in the lower airways, presumably by pulmonary blood flow. ^{35}S was present in the blood and urine within 5 minutes of exposure (the earliest time of sampling). More than half of the plasma ^{35}S was dialyzable; the remainder was bound to plasma proteins. Aeration in vitro of arterial and venous blood samples for 1 hour revealed a loss of one-fifth of venous blood radioactivity, but little to no loss from arterial blood. Urinary ^{35}S was 10 to 20 times that of blood. Renal excretion of ^{35}S exhibited a concentration-time relationship. No evidence of blood saturation of ^{35}S was present, with ^{35}S present at the end of the 180-minute recovery period.

b. Tissue Distribution

Balchum et al.[52] exposed dogs by tracheal cannula to radiolabeled sulfur dioxide at 1.8 to 148 ppm for 20 to 42 minutes. ^{35}S was found in every organ examined, with the highest levels in the respiratory tract (trachea, lungs, and hilar lymph nodes), but with activity found also in

the liver, spleen, and brain. Arterial blood ^{35}S increased during early exposure, promptly fell during recovery, presumably due to tissue diffusion and urinary excretion, while venous blood ^{35}S increased more slowly, peaking at 5 minutes into the recovery period.

In another study by Balchum et al.,[86] dogs were exposed by mouth and by nose to $^{35}SO_2$ at 1 to 141 ppm for 20 to 40 minutes. These dogs accumulated lower levels of ^{35}S in the trachea, lungs, hilar lymph nodes, livers, and spleens than did the dogs exposed by tracheal cannula. However, samples of the remaining abdominal viscera contained similar ^{35}S levels for both studies. This suggested that a high degree of sulfur dioxide absorption by the local mucosal tissue of the nose and mouth would protect the lungs from exposure (at least temporarily) and that uptake and redistribution by the blood would be similar whether the site of absorption was the nasal or oral mucosa or the lungs. Similar results were observed in both sets of studies for ^{35}S levels in venous blood, with these concentrations rising gradually during the exposure period and peaking during the recovery period. This phenomenon was suggestive of a gradual resorption of ^{35}S compounds from target tissue as blood levels and tissue levels started to equilibrate.

Frank et al.[51] reported that in dogs nasally exposed to $^{35}SO_2$ at 22 ppm for 30 and 60 minutes, ^{35}S appeared in whole blood the first sampling (5 minutes into the exposure period), became maximal near the end of exposure with no indication of saturation, and declined slowly during the 2-hour recovery period. The sulfur concentration was greater in the plasma than in the blood cells, and most of it (about two-thirds) was dialyzable with the nondialyzable remainder bound to plasma proteins (especially alpha-globulin). Urinary ^{35}S concentrations were 10 to 20 times that noted in the blood, with maximal excretion usually attained at or immediately after the end of exposure. Osmotic diuretics were without effect on excretion. The authors concluded that inspired sulfur dioxide reaching the lungs entered through the pulmonary capillaries, with distribution through the circulation ranging from 5 to 18 percent of the total dose.

Jonek et al.[26] exposed rats to radiolabeled sulfur dioxide ($^{35}SO_2$) alone and in combination with ammonia for 50 minutes. The concentrations of ^{35}S in the tissues examined 2 hours after exposure were less in animals exposed to sulfur dioxide-ammonia than in those exposed to sulfur dioxide only, with the greatest differences 24 hours after exposure ended. Two hours after sulfur dioxide exposure, the greatest ^{35}S was found in the serum, liver, kidneys, lungs, heart, gonads, and brain. Twenty-four hours postexposure, ^{35}S levels were still high in the kidneys (due to excretion) and lungs. Urinary ^{35}S in sulfur dioxide-exposed rats was approximately 20 percent less than that in sulfur dioxide-ammonia-exposed rats at 24 hours postexposure, with 13 percent and 24 percent of urinary ^{35}S, respectively, still present 4 days after exposure ended. The ^{35}S concentration was minimal in the brain and nonexistent in muscular tissue by autoradiographic analysis. In general, while the ammonia decreased the amount of inspired sulfur dioxide entering the animal, ^{35}S remained in most tissues examined for

the entire 4 days of recovery. Bystrova (cited in Jonek et al.[26]) found ^{35}S in rat tissues 11 days after exposure ended.

The widespread distribution of ^{35}S and thus SO_2 or its metabolites throughout all tissues in various animal species despite the mode of gas inhalation suggests the potential for toxicity to various organ systems. No attempt has been made to extrapolate the quantities of sulfur dioxide that human tissues would absorb under similar conditions. However, in human and animal studies, it is likely that ^{35}S would be distributed, retained, and excreted in a similar qualitative pattern, if not in similar quantities, in humans as in animals.

c. Metabolites of Sulfur Dioxide in Blood

(1) Human Experiments

Gunnison and Palmes[87] continuously exposed human subjects to sulfur dioxide at 0.3, 1.0, 3.0, 4.2, and 6 ppm for up to 120 hours. Findings indicated that each 1 ppm of the gas was converted to 1.1 nmoles of plasma S-sulfonate in both smokers and nonsmokers. The correlation between inhaled sulfur dioxide and plasma S-sulfonate was linear.

Gunnison and Benton[88] incubated human serum in vitro with sulfite at physiologic conditions. A steady-state equilibrium was established after 1 hour, with 81 percent of the sulfite still in free form. The absence in serum of certain proteins and other substances that are present in plasma precluded the extensive binding of sulfate to proteins and conversion to S-sulfonates normally occurring in human plasma and blood.

(2) Animal Experiments

Gunnison and Benton[88] continuously exposed rabbits to sulfur dioxide at 23 ppm for 14 and 62 hours. Rabbits exposed for 14 hours revealed immediate increases in serum and plasma cyanolytic sulfite of approximately 100 percent and 161 to 400 percent, respectively, above control levels. (Cyanolytic sulfite, a normal constituent of rabbit blood, was used to measure S-sulfonates by the addition of alkaline cyanide to serum and plasma samples.) Rabbits exposed for 62 hours showed increases in serum and plasma cyanolytic sulfite of approximately 300 percent and 310 to 323 percent, respectively, above endogenous levels, indicating that tissue equilibrium had not been reached after 14 hours. Absolute concentrations of cyanolytic sulfite were always greater in plasma than in the serum due to binding to plasma fibrinogen. Cyanolytic sulfite levels declined slowly, with the higher concentrations (62 hours) decreasing at a faster rate than the lower concentrations (14 hours). The apparent concentration-time relationship of oxidation of the cyanolytic sulfite by sulfite oxidase to S-sulfonates varied greatly among the rabbits, with half-lives from 30 minutes to 54 hours; in some rabbits, endogenous or preexposure levels were not attained more than 200 hours after sulfur dioxide exposure ended. The authors found that without the addition of alkaline cyanide, no free sulfate was present in

the blood, indicating that S-sulfonates are rapidly formed as a result of sulfur dioxide exposure. In a second experiment, the authors incubated rabbit plasma and sulfite at physiologic conditions. Most of the sulfite was nondialyzable (i.e., combined with large plasma proteins). The addition of an excess of sulfite to dialyzable and nondialyzable fractions of plasma resulted in two-thirds bound to the nondialyzable fraction, indicating that even at low sulfite levels in rabbit blood a dynamic equilibrium is established between free and bound sulfite, the latter forming plasma S-sulfonates.

Gunnison and Palmes[89] continuously exposed New Zealand white rabbits to sulfur dioxide at 10 ppm for 3.6 and 10 days. Exogenous plasma S-sulfonates immediately increased upon exposure, reaching an equilibrium at 3 to 5 days at approximately 225 percent above endogenous levels throughout the exposure period. Plasma sulfite was not detected in the samples. Immediately upon termination of exposure, S-sulfonate clearance proceeded rapidly and was proportional to the logarithm of the averaged normalized exogenous S-sulfonate concentration times clearance time, i.e., clearance (log c x t). Comparison of the clearance of exogenous S-sulfonate following sulfur dioxide inhalation and sulfite ingestion showed markedly slower clearance following inhalation than following ingestion, with a half-life for inhaled sulfur dioxide of 4.1 days and for ingested sulfite of 1.3 days.

d. Significance of Altered Sulfur Levels in Blood and Tissue

Gunnison and Benton[89] reported the lack of endogenous cyanolytic sulfite in humans compared to its presence in rabbits. The diversity observed between humans, rabbits, and other species regarding endogenous S-sulfonates may reflect a difference in sulfite oxidase activity among species, thereby determining the availability of endogenous sulfite for reaction with disulfide bonds of plasma proteins. The authors concluded that the evidence supports the supposition that sulfite entering mammalian blood during sulfur dioxide exposure forms S-sulfonate groups with constituents of the plasma, exclusively by sulfitolysis (i.e., the breakdown of sulfite and disulfite groups to form S-sulfonates). The reactions apparently attain a position of equilibrium with very close to 100 percent of sulfite in the oxidized or sulfonate form, provided that an excess of sulfite is not present. This interaction affords protection to many tissues of the body from high concentrations of sulfite. In addition, the same phenomenon may result in prolonged exposure to a very low level of sulfite, as sulfite oxidase indirectly causes the release of sulfite from S-sulfonate linkage, thus maintaining a low concentration of sulfite in the body.[89]

Shapiro[32] hypothesized that sulfite and the additional abnormal sulfur-containing metabolites may result in abnormal nucleic acids, amino acids, and proteins and may inhibit DNA synthesis, mitosis, and cell growth, resulting in the potential for carcinogenesis or co-carcinogenesis. Levels of sulfite oxidase and its rate of conversion of sulfur dioxide to plasma S-sulfonates were found to vary with species with rats the most efficient in the reaction, followed by rabbits, rhesus monkeys,

guinea pigs, and humans. The author postulated that variations in the activity of this enzyme account for the divergent resistance of these species to sulfur dioxide exposure.

At present, the consequences of the presence of increased levels of sulfur dioxide and its metabolites in the blood and peripheral tissues are unclear. Although the potential for untoward effects is suspected, there has been very little information suggesting that such is the case.

(1) Human Skin Reactions

Pirila[90] reported two cases of sulfur dioxide-induced skin allergies. The first subject developed skin lesions from chronic exposure to sulfur dioxide gas at unknown concentrations. Lesions disappeared while he was away from work or while wearing a gas mask at work. Diagnostic tests revealed that inhalation of sulfur dioxide at 40 ppm resulted in extreme skin reactions that were totally reversible after sulfur dioxide inhalation was discontinued. The second subject had a previous history of allergic response to sulfur-containing medications. When she was exposed to sulfur dioxide from a drainpipe near her home, extreme skin reactions resulted. Clinical tests revealed allergic response to a variety of sulfur compounds. After moving to a different residence, her allergic symptoms disappeared.

Pirila et al.[91] also reported a case of allergic skin reaction in a carpenter chronically exposed to sulfur dioxide at unknown concentrations from refrigerator machinery. Clinical tests revealed that sulfur dioxide inhalation at 10 ppm for 30 minutes induced a delayed, mild skin eruption. Repeated sulfur dioxide inhalation at 40 ppm for 10 minutes, interrupted by a few minutes of no exposure, resulted in no reaction the day of exposure but in severe skin eruptions the following day. The reaction subsided after several days and was completely reversible when the gas exposure was terminated.

(2) Animal Experiments

(a) Carcinogenesis

Peacock and Spence[71] repeatedly exposed LX mice (a breed chosen because they are known to be susceptible to the induction of lung adenoma by urethane) to sulfur dioxide at 500 ppm for 5 minutes per day, 5 days per week for 300 days. In mice surviving the 300 days of exposure, carcinoma of the lung was observed in females at approximately double the incidence observed in males or control animals. There was no difference in the incidence of hepatoma (hepatocarcinoma) and lymphocytosis in exposed animals compared with controls. There was an association between sulfur dioxide exposure and persistent lymphatic engorgement, alveolar hyperplasia, and progression to neoplasia. Malignancy was observed in large tumors only and presumably occurred as a progressive development in a tumor which had already reached about 5 mm (implying that the tumor had been growing for some time). In addition, these large tumors appeared at an earlier age in the animals exposed to sulfur dioxide than in non-exposed animals, suggesting that in mice repeated, intermittent long-term

exposures of sulfur dioxide promoted the sequence of events resulting in the growth of spontaneous lung tumors. While sulfur dioxide may be considered tumorigenic for spontaneous primary lung tumors, the evidence does not justify the classification of sulfur dioxide as a chemical carcinogen, i.e., that the gas itself causes malignancy.

(b) Hepatic Toxicity

Murphy et al.[92] continuously exposed rats to sulfur dioxide at 58 ppm for 20 hours. The animals exhibited significant increases over controls in the ratio of liver weight to body weight ($p < 0.01$) and in levels of liver alkaline phosphatase (an enzyme whose levels tend to increase in cases of biliary damage), with a nonsignificant decrease from controls in adrenal gland weight. In addition, the animals experienced dyspnea, eye and nose irritation, and generalized debilitation. The authors concluded that inhalation of sulfur dioxide resulted in damage to extrapulmonary sites, in addition to the usual pulmonary effects observed. No concentration-time relationship could be discerned from the data presented and the reversibility of the effects was not addressed.

(c) Effects on Reproduction

Shalamberidze and Tsereteli[93] intermittently exposed sexually mature female albino rats to sulfur dioxide alone at 0.6 and 1.9 ppm and to a combination of sulfur dioxide and nitrogen dioxide at 0.97 ppm and 0.64 ppm, respectively, for 12 hours per day for 3 months. Sulfur dioxide alone at 0.6 ppm had no effect on the estrous cycle; the organs examined (ovaries, uterus, pituitary, thyroid, and adrenals) displayed no pathomorphologic changes, and reproductive functions were not disturbed. Exposures to the higher level of sulfur dioxide (1.9 ppm) and to the gases in combination caused a prolongation of the cycle due to an increase in the interestrous period and a decrease in the monthly number of estrous cycles, especially normal cycles. The most pronounced effects were observed during months 2 and 3 of the exposure, indicating a concentration-time relationship. Diestrus was particularly prolonged and estrus became less frequent and more prolonged. All estrous-cycle disturbances were reversible by 7 months after exposure ended. Histopathologic changes were observed in the pituitary, adrenals, thyroid, ovaries, and uterus, mainly in the form of circulatory disturbances (e.g., hyperemia, stasis, and hemorrhages). The number of primordial follicles decreased in the ovaries; the thyroid gland became depleted in interfollicular connective tissue; and the uterus became depleted in glandular epithelium. In addition, sporadic mild degenerative phenomena were observed in the adrenals, ovaries, and uterus. The capacity to become impregnated was not adversely affected. However, evidence of disturbed reproductive capacity was present: the size of litters and birth weight were significantly decreased compared to controls ($p < 0.001$), and there was a subsequent lag in weight gain in the offspring compared to controls ($P < 0.001$). The evidence suggested that the higher levels of sulfur dioxide alone or in combination with nitrogen dioxide adversely affected the intrauterine development of rat fetuses.

H. CONCENTRATION-TIME RELATIONSHIPS

In cases of sulfur dioxide exposure, the effect of Ct (the product of concentration times exposure time) has not been extensively investigated. However, there is evidence in both human and animal studies that the immediate irritant and respiratory effects of sulfur dioxide exposure are primarily concentration-dependent and, except near lethal concentrations, probably completely reversible.[7-12,14,21] Moreover, as exposure continues, several acute effects on respiratory capacity are diminished, suggesting an adaptive response.[15,19,22,33] Such acclimation has been reported in several species, including humans.

It is also suspected that exposure to a given concentration of sulfur dioxide will have different effects, depending on whether the exposure period was prolonged and continuous or short and intermittent for the same total time and thus the same Ct value.

1. Lethality

a. Accidental Human Overexposure

Lethality data for humans are based on studies of industrial accidents, for which neither the sulfur dioxide concentrations nor the exposure times were known.[7-11] At these lethal levels, concentration was apparently the primary determinant in immediate death. Among those who initially survived the exposure, the incidence of delayed death or irreversible pulmonary damage seemed to be related to both concentration and time.

b. Animal Studies

LCt_{50} values from three studies[8,14,32] (shown in Table 5) suggest that for mice the lethal effects of sulfur dioxide exposure depend mainly on gas concentration. As sulfur dioxide concentrations decrease, the associated LCt_{50} values generally increase. The data shown for guinea pigs, however, suggest that the concentration-time relationship may be more important for this species, because at either 130 or 1000 ppm, the LCt_{50} was about 20,000 ppm hours. The results from a single study, however, can be viewed only as preliminary evidence of this possibility.

2. Immediate, Reversible Irritant Effects

In general, each of the irritant effects of sulfur dioxide is associated with specific threshold of the gas concentrations[22] (refer to Table 3). These effects have been demonstrated in both humans and animals within the first few breaths of the gas at the threshold concentration for that effect. These responses do not tend to increase with continued exposure but instead usually subside or disappear after several

TABLE 5
LCt_{50} Values from Animal Studies

Species	SO_2 Concentration, ppm	Time	LCt_{50} ppm hours	Reference
Mice	1948	9.8 min	318	14
	1375	37.5 min	859	
	1337	10.0 min	223	
	878.6	217.0 min	3,178	
	611	59.0 min	601	
Mice	1350	10.0 min	225	8
	610	1 hour	610	
	340	6 hours	2,040	
	130	24 hours	3,120	
Mice	1000	4 hours	4,000	32
	150	847 hours	127,500	
Guinea Pigs	1000	20 hours	20,000	
	130	154 hours	20,020	

minutes of exposure, as observed in studies of both humans and animals.[15,19,22]

Rapid adaptation to the irritant effects of sulfur dioxide at concentrations up to 25 ppm has been observed in humans.[24,72]

3. Immediate Respiratory Effects

In general, immediate respiratory effects of exposure to sulfur dioxide have been influenced primarily by the concentration of sulfur dioxide.[15,19,33,56] Frank et al.[19] and Frank[15] noted PFR changes in humans continuously exposed to sulfur dioxide at 1, 5, and 13 ppm for 30 minutes. At 5 and 13 ppm, PFR increased to one-half the peak level within 45 seconds of exposure, reaching a maximum level at 5 to 10 minutes with no further increases with time. From 10 to 30 minutes of exposure, the effects spontaneously declined from their peak levels but remained above control values. Changes at 13 ppm were significantly greater than those at 5 ppm.

Sim and Pattle[25] exposed subjects to sulfur dioxide at 5.1 to 51.2 ppm for 10 to 60 minutes per day (at 24-hour intervals and two exposures per week) for various numbers of exposures. The changes in PFR were similar to those observed by Frank et al.[19] and Frank,[15] with peak changes in respiratory effects occurring in the first few minutes of exposure and subsiding thereafter.

These studies help demonstrate the general absence of a Ct relationship for immediate respiratory effects and the predominance of concentration as the primary determinant of these effects.

4. Continuous versus Intermittent Exposure

It is difficult to compare the effects of prolonged and continuous exposure with the effects of short and intermittent exposures at the same or similar Ct levels. In order to make valid comparisons, data should come from the same animal model in the same study, at the same sulfur dioxide concentration, for the same number of total exposure hours, and with the authors using the same toxicity parameters for all exposure groups. No available data even approach these criteria.

It has been suggested by Goldring et al.[73,94] that intermittent exposure allows for the restoration of damaged tissue during the period of nonexposure, thereby reducing the overall effect of the Ct relationship observed in continuous exposure.

There is indirect evidence in epidemiologic data that intermittent exposure to moderate concentrations of sulfur dioxide at enormous Ct levels is without severe health consequences. Kehoe et al.[24] examined workers who were intermittently and chronically exposed to sulfur dioxide at reported daily mean concentrations of 20 to 30 ppm for up to 19 years. The Ct values for some of these workers approached 1,000,000 ppm·hours. Although numerous adverse effects were reported, none was judged serious by the criteria used in this study. This suggested that the intermittent periods of nonexposure were sufficient for repair of damage,

if any, to pulmonary tissue. There are, however, no data on continuous exposure to these sulfur dioxide levels; thus, it cannot be assumed that intermittent exposure necessarily results in fewer or less severe health effects than does continuous exposure.

5. Neurologic Effects

The Ct relationship for neurologic effects of sulfur dioxide appears to be related solely to gas concentration; below the threshold concentration for sensory recognition, no effects occurred despite the duration of exposure.[5,16,17]

6. Tracheobronchial Clearance

Tracheobronchial clearance exhibited an interesting variety of Ct relationships. In one study, no sulfur dioxide-induced changes in tracheobronchial clearance were observed below 300 ppm; above 300 ppm, clearance was decreased, with maximum effects (sixfold increases in clearance time) observed at 557 to 713 ppm.[79] In another study,[80] clearance was inversely proportional to the concentration multiplied by time to the fourth power (clearance $\propto \frac{1}{C \times t^4}$). Similar findings were observed in additional studies.[81,85]

7. Tissue Distribution

Tissue distribution of sulfur dioxide and its metabolites was primarily dependent on time, while tissue concentration appeared to be dependent on both time and concentration.[44,84,85] Excretion of sulfur dioxide and its metabolites (primarily in the urine) from tissues and blood was also dependent on concentration and time, with time the predominant factor.[95] The urinary clearance appeared to be proportional to the product of the logarithm of the concentration multiplied and time, i.e., clearance $\propto \log_{10} C \times t$.

8. Other Factors Influencing the Ct Relationship

In general, while the duration of exposure to sulfur dioxide at any given concentration is an important determinant of toxic effects, at equivalent Ct levels, concentration appears to be the primary factor. The effects of exposure to higher concentrations for shorter periods will thus be more pronounced than the effects of lower concentrations for longer periods.[56] However, the inference should not be made that long-term, low concentrations of sulfur dioxide produce no adverse effects.[28] Adverse effects do occur; the severity and reversibility of these effects are in part determined by the Ct relationship, but there are other determinants.

As discussed previously, the sulfur dioxide concentration necessary to induce immediate respiratory effects is altered by the mode of gas inhalation, the rate of breathing or minute volume, and the addition of various aerosols and gases to the sulfur dioxide. Mouth breathing reduces the concentration of sulfur dioxide needed to produce immediate

respiratory responses and increases the degree of the effects.[15,19,22,28,48,49] Increased respiratory rates (and consequently increased minute volumes) have also been observed to affect the immediate respiratory effects.[30] A number of authors[15,19,65] have reported that a variety of other gases and aerosols have enhanced the immediate respiratory effects of sulfur dioxide exposure in both humans and animals.

All these factors may alter the Ct relationship for a particular concentration of sulfur dioxide and its concomitant immediate respiratory effects. In general, however, the immediate respiratory effects are predominantly concentration dependent (with time as an important secondary factor) and are quickly reversible.

V. SUGGESTED FOLLOW-ON WORK

The effects most likely to affect adversely the performance of soldiers in training or combat are the immediate respiratory effects--including copious lacrimation, irritant effects, intense bronchoconstriction, hemoptysis, and epistaxis--and irritancy of such magnitude that it results in a desire to leave the environment.

The available literature provided data sufficient to determine threshold limits for a variety of the immediate irritant, respiratory, and neurologic effects of sulfur dioxide exposure in humans (refer to Tables 1 and 3). Examples include:

- Lowest level detectable (probably by taste)
- Lowest level of odor perception and irritation of mucosa
- Lowest level causing suppression of dark adaptation and decreased light sensitivity
- Lowest level causing elevation of optical chronaxy and disruption of alpha rhythms
- Lowest level causing coughing, throat irritation, anterior chest irritation, increased salivation, and bronchoconstriction
- Lowest level considered extremely disagreeable
- Lowest level causing extreme irritation of eyes, nose, and throat, epistaxis, copious lacrimation, sneezing, and hemoptysis.

Information in the literature was less adequate for the determination of additional threshold limits, usually because the data were derived from early studies and/or have not been confirmed by additional studies. Examples include:

- Lowest lethal level regardless of exposure time
- Lowest levels causing death within specified times
- Lowest level causing irreversible effects on respiratory function or pulmonary histology.

Suggested follow-on studies include:

- Additional studies on the neurologic effects of sulfur dioxide exposure and an estimate of the role these effects can play in the alteration of judgment, decision-making, and combat or training work efficiency

- Estimates of the times at which copious lacrimation, severe bronchoconstriction, and additional irritant and pulmonary effects will begin to diminish the work efficiency of soldiers in training or combat

- Lethality studies on animals for short-term, high-level exposures. Since guinea pigs and monkeys closely resemble humans in the response to sulfur dioxide exposure, the use of these species is suggested

- For each of the irreversible effects that have been reported in both humans and animals, animal studies should be conducted to determine the concentration and/or time necessary for each effect to occur

- Further studies of exposure to sulfur dioxide in combination with other gases found to be present in armored vehicles and with various aerosols at a wide range of particle sizes

- Studies to evaluate the effects of sulfur dioxide-induced neurologic changes on the performance of certain tasks

- Studies of the effects of various levels of physical stress on baseline human physiologic values including respiratory rate and volume, airflow resistance, and other pulmonary function parameters.

Further studies should be performed on animals that have not been markedly altered from their normal physiologic states by anesthesia, surgery, or physical manipulation. It has been demonstrated that these types of preparations may greatly affect the study results.[29,34-38] Suggested animal models include the monkey and the guinea pig. Exposure levels in the studies should approach the most severe anticipated military operational conditions as closely as possible, i.e., 1 hour per exposure, six exposures per day for 14 days.

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APPENDIX A

REVIEW OF THE LITERATURE

A. HUMAN STUDIES

1. Human Experiments

a. Pulmonary Effects

• Frank NR: Studies on the effects of acute exposure to sulphur dioxide in human subjects. Symp Air Pollut, Proc Soc Med 57:1029-1033, 1964

Review:

This paper summarizes a group of studies of the effect of sulfur dioxide (SO_2) on the airway caliber of the lungs, measured in terms of pulmonary flow resistance (PFR). Frank studied changes in PFR in human volunteers exposed to SO_2 at 1 to 13 ppm in a volume-displacement body plethysmograph.

To establish dose-response relationships, 11 subjects were exposed by mouth to SO_2 at 1 ppm (range 1-2), 5 ppm (range 4-7), or 13 ppm (range 10-16). Exposures lasted 10 to 30 minutes and were at least 1 month apart. The esophogeal catheter technique was used to measure PFR (in cm H_2O per liter per second).

Sodium chloride (NaCl) aerosol was added to the inspired air in the next series of experiments to determine if it would potentiate the bronchoconstrictive effects of SO_2. NaCl aerosols (average concentration, 18 mg/m^3; particle size, 0.04-0.15) were administered in combination with SO_2 at 1 to 2, 4 to 6, and 14 to 17 ppm (average concentrations were not given) for 30 minutes. PFR measurements were made using the same techniques as for the dose-response experiments above. In one experiment, subjects were exposed to SO_2 by itself for 10 minutes followed by 15 minutes of breathing air, followed by a 10-minute exposure to SO_2 in combination with aerosol. Other subjects were exposed to the same conditions in reverse sequence (SO_2 with aerosol first, SO_2 alone last).

In another series of experiments, Frank compared changes in PFR when subjects breathed SO_2 by nose or by mouth, at concentrations of 15 and 29 ppm for 25 minutes.

The last series of experiments measured SO_2 uptake in the airways. Seven subjects were exposed to 12.4 ppm SO_2 by nose for about 30 minutes. Gas was collected in ion chambers during inspiration, expiration, or both stages of the cycle alternately. Sampling sites included 2 cm in front of the nose in the path of the airstream and in the oropharynx as far back as could be tolerated. Generally 8 to 10 minutes of intermittent sampling was required per analysis. SO_2 was measured conductometrically with a vibrating reed electrometer.

In the dose-response experiments, only one subject, a smoker whose control PFR was high (3.42 cm H_2O per liter per second compared with the others' range of 0.80-1.62), showed an increase in PFR in response to the 10-minute, 1 ppm exposure. When exposed to SO_2 at 5 and 13 ppm for 10 minutes, the subjects showed an average increase in PFR of 39 and 72 percent, respectively, above control values. There was no relationship between changes in flow resistance and the amount or duration of cigarette smoking, nor was there a correlation between the time to onset of irritant symptoms (e.g., coughing, throat and upper chest irritation) and the changes in PFR due to bronchoconstriction. These symptoms reached a peak within the first few breaths of SO_2 and then subsided at a time when the bronchoconstrictive effects were still intensifying.

In five subjects exposed to SO_2 at 4 to 15 ppm for 30 minutes (no other details given), changes in the PFR occurred within the first minute of exposure and were maximal at 5 to 10 minutes. Thereafter, although the PFR remained elevated above control levels, it diminished to below the maximal response after the first 10 minutes, suggesting an adaptation reflex. The average PFR for the group remained elevated above control levels though reduced from its peak level 15 minutes after the end of exposure to both 5 and 13 ppm of SO_2. However, in several subjects, after a 15-minute recovery period, the PFR was not significantly elevated over preexposure levels. There were no consistent changes in pulmonary compliance, tidal volume, breathing frequency, or pulse rate. The functional residual capacity increased slightly during exposure to 13 ppm.

In the next series of experiments, exposures to SO_2 in combination with NaCl aerosol were found not to have any potentiating effect in the production of PFR changes.

In the nose-versus-mouth experiments, PFR increased significantly above control levels in 9 of 12 experiments in which SO_2 was administered by mouth, but in only 3 of 12 subjects exposed by nose. Symptoms of cough and chest irritation were far less common in subjects breathing SO_2 by nose than by mouth. Frank concluded that SO_2 penetrated farther along the airway when breathing was by mouth.

In the SO_2 absorption experiments, the author demonstrated that about 99 percent of the SO_2 administered at 12.4 ppm was absorbed by the nasopharyngeal mucosa during inspiration, and the rate of absorption did not change over the 30-minute exposure period. Expired air from the lungs was essentially free of SO_2 at the level of the pharynx; there was some desorption of SO_2 from the nasal mucosa, because air expired from the nose contained about 2 ppm.

Analysis:

The results of some of the experiments would have been more useful if average SO_2 concentrations had been given (as in the NaCl aerosol experiment) or if the broad concentration range had been narrowed into smaller ranges (as in the experiment where SO_2 was administered at 4-15 ppm to determine changes in pulmonary function values).

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• Sim VM, Pattle RE: Effect of possible smog irritants on human subjects. J Am Med Assoc 165(15):1908-1913, 1957

Review:

Sim and Pattle measured pulmonary flow resistance (PFR) in an unspecified number of healthy young male volunteers (aged 18-45 years) exposed to SO_2, sulfuric acid mist (H_2SO_4), and various aldehydes. SO_2 gas was administered to some subjects individually by oxygen-type masks (1.34-80 ppm for 10 minutes) and to other subjects in groups in a 100-m^3 chamber (1.0-23.1 ppm for 60 minutes). No group was exposed more than twice in a week, and there was at least 24 hours between exposures. No restrictions were placed on the activities (including smoking and walking) of the subjects exposed in the chamber. Blood pressure, pulse rates, and respiration rates were recorded before, during, and after exposure. FKGs were taken on a number of subjects in each exposed group and PFR levels were measured by means of an Ainsworth interrupter, a device that measures transpulmonary pressure at a time of no gas flow (end of inspiration or of expiration). SO_2 was analyzed by acidification with hydrogen peroxide and titration with base. Unexposed men were used as controls.

The results of exposure to SO_2 and H_2SO_4 mist are shown in Table A-1. Although PFR values were not reported, the authors stated that with SO_2 dosages of approximately 300 ppm minute little change in PFR was noted with the exception of an occasional rise of more than 20 percent of the preexposure level; however, specific data for SO_2 levels and PFR values were not given. Such rises were accomplished in some, but not all, subjects by clinical findings of moist rales bilaterally in the upper anterior chest (i.e., anteriorly over the large bronchi). At doses above 8.5 ppm for 60 minutes (approximately 512 ppm min), PFR was increased significantly above preexposure levels in half the exposed subjects, whether the gas was administered by mask or in the chamber. The increase in PFR was maximal within the first 10 minutes of exposure, with little further change by the end of 1 hour. Rhinorrhea and lacrimation accompanied exposures at these levels, and clinical examination of the chest revealed the presence of high-pitched musical rales and prolongation of expiration. Moist rales were commonly heard in the more peripheral lung fields at the high dose levels. Changes in pulse rate, respiration rate, tidal or supplemental air volumes, vital capacity, maximum breathing capacity, and blood pressure were observed infrequently.

Burning magnesium oxide ribbon into the atmosphere of the chamber diminished the irritant and bronchoconstrictive effects of SO_2 exposure, and when ammonia was used in sufficient concentration, there was almost immediate relief of these symptoms.

TABLE A-1

Results of Exposure to Sulfur Dioxide
and Sulfuric Acid Mist

Agent	Method	Concentration, ppm	Duration, min	No. of Exposures	Effects
SO_2	Mask	1.34-80	10	264	Bronchoconstriction, rales in chest
SO_2	Chamber	1.0-23.1	60	330	Bronchoconstriction, rales in chest
SO_2 (with NH_3[a] after 50 min)	Chamber	9.9	60	10	Smell and bronchoconstrictive effect disappeared when ammonia was liberated
SO_2 (with MgO[a] after 50 min)	Chamber	6.0	60	10	Smell and bronchoconstrictive effect disappeared when MgO was dispersed
H_2SO_4 (10 N, 1um diameter)	Mask	1.0-9.6	10	183	Coughing, some bronchoconstriction, rales
H_2SO_4 (10 N, 1um diameter)	Chamber	0.7-9.6	60	316	Coughing, some bronchoconstriction, rales
H_2SO_4 (4 N, 1.5um diameter)	Chamber	2.8-9.2	30-60	40	More irritant than dry mist at same concentration
H_2SO_4 (with NH_3[a] at end of exposure)	Chamber	0.7-9.6	---	356	Irritation disappeared when ammonia was liberated at end of acid mist exposure
H_2SO_4 (with 9.9 ppm magnesium oxide after 20 min)	Chamber	7.3	30	10	Irritancy not diminished when magne oxide was dispersed

[a]Concentration not given.

Note: Table adapted from Sim and Pattle. J Am Med Assoc 165(15): 1910, 1957.

A coincidental observation made during the study was that the authors themselves, who participated as subjects, developed what seemed to be an increasing sensitivity to both SO_2 gas and H_2SO_4 mist. One of them developed a persistent bronchitis over the 10-month period of repetitive exposures. Subsequent exposures to either the gas or the mist resulted in exacerbation of the symptoms and a period of coughing and wheezing. The other author also developed chest symptoms and, after the final SO_2 exposure, complained of 4 days of persistent wheezing.

It was also reported that when wet mist of H_2SO_4 at about 5.1 ppm was used, the irritancy was greater than with dry mist at double the concentration and similar to that observed in subjects exposed to SO_2 at higher (unspecified) concentrations. PFR levels were increased in most cases by more than 20 percent; however, actual data were not presented.

Analysis:

Although various cardiovascular measurements (blood pressure, pulse rates, EKGs, and PFR levels) were collected during this study, only general clinical findings were reported for each exposure group. When increases in PFR levels were reported, no quantitative information was given on the magnitude or duration of those increases; thus direct comparisons with similar studies are not possible. It is also unfortunate that the range of SO_2 concentrations tested was so broad. Bronchoconstriction and chest rales were reported for subjects exposed to SO_2 by mask at 1.34 to 80 ppm for 10 minutes and by chamber exposure at 1.0 to 23.1 ppm for 60 minutes. It would be useful to know the narrower level of exposure and number of exposures at which those clinical effects were detected.

It is also noted that because of the frequency of exposures, clearance of S-sulfonates from the body may take several days to weeks, depending on the dosage. The 24-hour recovery period in the present study was probably too short for complete SO_2 clearance between exposures; therefore, SO_2 metabolites would tend to accumulate in the volunteers with each new exposure, possibly altering the results in subsequent exposures.

In the chamber experiments, allowing the subjects to move about and to smoke introduced variables that may have greatly altered the test results.

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• Frank NR, Amdur MO, Worcester J, Whittenberger JL: Effects of acute controlled exposure to SO_2 on respiratory mechanics in healthy male adults. J Appl Physiol Lett 17(2): 252-258, 1962

Review:

Frank et al. studied the effects of exposure to SO_2 at 1, 5, and 13 ppm on respiratory mechanics in healthy adult males.

Eleven volunteers (average age: 36 years; range: 22-56 years) were exposed to SO_2 by mouth while seated in a volume-displacement body plethysmograph. Various measurements were made during the control period during which the subjects breathed room air. Subjects were then exposed to SO_2 at about 1, 5, and 13 ppm (ranges: 1-2, 4-7, and 10-16 ppm, respectively) for 10 to 30 minutes. The concentration of SO_2 in the chamber was monitored by the electroconductivity method of Thomas and Abersold. In some instances, the physiologic measurements were begun 30 seconds after exposure, and generally required approximately 1 minute to complete (such measurements were referred to as changes at 1 minute). In all instances, measurements were made after 5 and 10 minutes of exposure with the exposure remaining uninterrupted during these measurements. Measurements were also taken 15 minutes after cessation of exposure. The authors waited at least 1 month before repeating an experiment on the same subject, since frequent prolonged SO_2 exposure might have affected the degree of response.

Esophageal pressure was measured by means of a tube with a balloon passed to the lower part of the esophagus. Transpulmonary pressure was measured by an inductance manometer. Volume was measured by a Krogh spirometer mounted on the plethysmograph. The rate of volume change (flow rate) was obtained by two methods: electrical differentiation of the volume signal and analysis of photographs of pressure-volume images appearing on an oscilloscope from which tracings were made. Pulmonary flow resistance (PFR) was calculated either from the photographs or from the oscillograph tracings. In both instances, the PFR value represented an average flow resistance for inspiration and expiration occurring in the midtidal range of volume. Values for both pulmonary compliance (ratio of change in volume to change in transpulmonary pressure) and PFR were averages, based on sets of 10 breaths. The end expiratory gas volume or functional residual capacity (FRC) was measured. Measurements of PFR and FRC were calculated for the control period, the SO_2 exposure period (10 minutes), and the recovery period for 1, 5, and 13 ppm SO_2 (Table A-2).

In some experiments, the timed vital capacity (TVC) was also measured, as well as the maximum expiratory flow rate (MEFR), for which the Wright flowmeter was used. The pulse rate or heart rate (HR) was counted in 11 subjects, using EKG limb leads. Statistical procedures included the Student's and paired t-test.

The resulting data from trials in which SO_2 exposure was prolonged to 30 minutes in order to determine the effect of time of PFR changes are shown in Table A-3. While the authors believed the number of trials was insufficient to yield definitive data, two tentative conclusions were made. At 1 ppm SO_2, prolongation of exposure to 30 minutes did not increase the likelihood of a significant rise in PFR. At the higher levels of SO_2 exposure (4 and 5 ppm), there appeared to be no progressive increase in PFR with time. Data from a single subject, subject 11, who breathed 15 ppm SO_2 for 30 minutes, gave evidence that a diminution in response could occur. This subject had a very high PFR value during the control measurement, which reached a maximum in 5 minutes and then decreased for the remaining 25 minutes of exposure. During the recovery phase (15 minutes after SO_2 exposure ended), the average PFR for each group remained elevated above control PFR values. Subject 11 recovered rapidly; 15 minutes after SO_2 exposure was terminated, his PFR was again comparable to his preexposure level.

FRC showed no consistent trend during exposure to 1 and 5 ppm SO_2. However, at 13 ppm SO_2 there was an increase in FRC which averaged 0.03 liters (p 0.01); this was unaccompanied by changes in either end-expiratory esophageal pressure or pulmonary compliance.

Pulmonary compliance was slightly decreased. The pulse rate or heart rate (HR) was unaffected by exposure to SO_2. During the control state, HR averaged 84 beats per minute (range: 68-111). After 1, 5, and 10 minutes of exposure to SO_2 at 4-15 ppm, the mean HR values were 84 (range: 72-108), 87 (range: 72-111), and 86 beats per minute (range: 69-114), respectively. The average value 15 minutes into the recovery phase was 88 beats per minute (range: 72-117).

In 10 subjects, the authors attempted to compare changes in PFR, MEFR measured with the Wright flowmeters, and TVC. TVC could not be measured in two group members due to coughing. All 10 subjects showed increases in PFR during SO_2 exposure (based on measurements performed just before MEFR and TVC), while nine subjects showed a reduction in MEFR ($p < 0.01$). The increase in PFR was 89 percent, whereas MEFR decreased by only 7 percent. The values for TVC (1-second expiratory capacity and vital capacity) were generally lower during exposure, but changes expressed as percentages were negligible. Subjective symptoms were usually elicited by 4.5 ppm SO_2 or higher and included coughing, a sense of irritation in the throat and anterior chest, and increased salivation.

The degree to which PFR increased during SO_2 administration was related to the concentration of the gas and varied with time of exposure to a limited degree in that the changes caused by 5 or 13 ppm SO_2 were only about half as great after 1 minute as they were after 5 to 10 minutes.

Analysis:

The statistical design and procedures used in this study were appropriate, and the results are assessed to be scientifically valid. The authors' limited experiments with exposures lasting 30 minutes

TABLE A-2

Average PFR and FRC Values Before, During, and After 10-Minute Exposure to SO_2

Condition	PFR ($cm\ H_2O$/liter/sec) and FRC (liters)					
	1 ppm SO_2		5 ppm SO_2		13 ppm SO_2	
	PFR	FRC	PFR	FRC	PFR	FRC
Control (preexposure)	1.57	3.93	1.31	4.10	1.74	4.15
Exposure (at 10 min)	1.58	3.63	1.82[a]	4.03	2.99[b]	4.45
Recovery[c]	1.63	3.76	1.62	4.04	1.96	4.8
Number of subjects	11	5	11	10	11	10

[a] $P < 0.01$ for differences between control and exposure values.

[b] $P < 0.001$ for differences between exposure values and control or recovery values.

[c] Measured 15 minutes after cessation of exposure.

Note: Adapted data from Frank et al. J Appl Physiol 17(2):252-258, 1962.

TABLE A-3

Time Course of Changes in PFR During 30-Minute SO_2 Exposure and During Recovery

Number of Subjects	SO_2 Concentration, ppm	Exposure PFR(cm H_2O/liter/sec)					Recovery PFR, 15 min
		Control	5-min	10-min	20-min	30-min	
5	1	1.41	1.36	1.36	1.32	1.44	1.47
5	4	1.02	1.43	1.50	1.14	1.32	1.72
1	5	0.85	1.38	1.23	1.24	1.05	1.08
1	15	3.34	6.09	5.82	5.97	4.69	3.38

Note: Table adapted from Frank et al. J. Appl. Physiol. 17(2):252-258, 1962.

provided some evidence that PFR is not likely to continue increasing with time. However, the authors did not comment on the possible effects of increased concentrations of SO_2 (i.e., greater than 15 ppm) on PFR.

This study demonstrated that MEFR may be slightly affected when PFR is increased as little as 1.16 cm H_2O per liter per second (range of increase: 0.03-2.22). The changes in TVC were not significant for the smaller group of subjects used. A possible explanation for the relative insensitivities of the MEFR and TVC was that these measurements were preceded by a maximal inspiratory effect, whereas the PFR was measured during quiet breathing. While the reduction in MEFR was significant, that for TVC was not; this may have resulted from using different subjects for each of the studies. The symptomatic responses proved to be unreliable indices of changes in respiratory mechanics. On the contrary, coughing and sense of irritation in the throat and chest tended to subside after 5 minutes of SO_2 exposure when the increase in PFR was maximal.

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• Nadel JA, Salem H, Tamplin B, Tokiwa Y: Mechanism of bronchoconstriction during inhalation of sulfur dioxide. J Appl Physiol 20(1):164-167, 1965

Review:

Nadel et al. studied human subjects and cats to ascertain the mechanism of bronchoconstriction during SO_2 inhalation, e.g., reflex action and direct effect of SO_2 on bronchial smooth muscle.

Airway resistance and thoracic gas volume were measured in seven human subjects (age range: 27-40 years). Airway resistance was converted to airway conductance by determining its reciprocal. All data were calculated as the ratio of airway conductance to thoracic gas volume; this allowed for variations in lung volume during the test. The subject, wearing a nose clip, sat in the closed plethysmograph and panted briefly while four consecutive control measurements were obtained for airway resistance and thoracic gas volume. The subjects were then exposed to 4 to 6 ppm SO_2 for 10 minutes while measurements of airway resistance were made once every 15 to 30 seconds. Reported control values of the airway conductance to thoracic gas volume ratio were the averages of four consecutive measurements. SO_2 concentration was determined by using an SO_2 analyzer and a modification of the West and Gaeke method. Twenty-four hours after the control experiments, subjects received 1.2 to 1.8 mg of atropine sulfate subcutaneously, and were then exposed to SO_2 20 minutes later.

In the second series of experiments, 11 cats were each tracheostomized at either of two sites for delivery of SO_2 (for a single inflation of the lungs) by means of a cannula directed downward to the lower airway and lungs or upward to the upper airway and out through the mouth. Airflow was measured with a Fleisch pneumotachograph and Statham differential strain gauge, and a volume signal was obtained by electrically integrating the airflow signal. Pulmonary resistance (R_L) was measured by a method of electrical subtraction. Blood pressure was measured by means of catheters inserted into the femoral arteries and attached to a Statham strain gauge. Nerve impulse conduction in the cervical vagosympathetic nerves was blocked by brass thermodes(at 0° C) through which cold saline was circulated. The concentration of SO_2 administered was not measured.

Results with human subjects showed that inhalation of 4 to 6 ppm SO_2 decreased the ratio of airway conductance to thoracic gas volume in each of the seven subjects by a mean of 39 percent ($p < 0.001$) compared to control values (indicating a high degree of airway resistance). During SO_2 exposure, most of the subjects experienced coughing (which decreased with time) and a sensation of irritation in the pharynx and substernal area. The time to onset of changes in airway conductance varied from 10 seconds to 4 minutes. Subjects who were injected subcutaneously with atropine (1.2-1.8 mg) and then exposed to SO_2 experienced no significant change

from controls in the ratio of airway conductance to thoracic gas volume, and atropine did not affect the incidence of coughing or sensation of irritation in the pharynx and substernal area.

The results for cats indicated that SO_2 delivered into the lower airways and lungs during a single inflation produced a mean increase 246 percent above control values ($p < 0.05$) in the R_L measurements of the 11 cats. R_L usually began to increase during the first breath after SO_2 exposure and returned to normal limits within 1 minute. Repeated inhalations with intervals of 10 minutes usually continued to increase R_L. Changes in femoral arterial blood pressure were slight and inconsistent. The same dose of SO_2 delivered to the upper airways increased R_L in seven of eight cats by a mean increase of 331 percent above control values ($p < 0.05$). With the cooling of the cervical vagosympathetic nerves, R_L did not increase significantly in response to SO_2 administration whether delivered to the lower or upper airways. After rewarming the vagosympathetic nerves, SO_2 administration again resulted in the usual increase in R_L. After injection of atropine sulfate into the femoral vein (1.0 mg/kg), introduction of SO_2 into the upper or lower airway resulted in no change in R_L.

Analysis:

The authors stated that these results indicate that brief exposure to low concentrations of SO_2 causes bronchoconstriction that depends on intact motor parasympathetic pathways; their studies provided no evidence that local stimulation of smooth muscle is responsible. However, they had not studied the effect of very high concentrations or prolonged exposures of SO_2, which may cause erosion of the epithelial lining, submucosal edema, or other changes in the lungs. The rapidity of the responses and their reversal suggested to them that changes in smooth muscle tone probably caused the bronchoconstriction. These experiments indicate that the receptors occur in the upper as well as lower airway, since lower airway constriction in cats also occurred when only the upper airway was exposed to SO_2. The results of this study also indicate that the efferent limb of the bronchoconstrictor reflex after SO_2 inhalation was through the vagus nerves.

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• Snell RE, Luchsinger PC: Effects of sulfur dioxide on expiratory flow rates and total respiratory resistance in normal human subjects. Arch Environ Health 18:693-698, 1969

Review:

Snell and Luchsinger studied the acute effects of 0.5 to 5 ppm SO_2 on expiratory flow rates and total respiratory resistances in human subjects in a single-blind study.

In the first series of experiments, nine volunteers (aged 20-40 years) inhaled each of four test samples for 15 minutes through a mouthpiece. These samples were:

1. aerosols of distilled water or normal saline
2. SO_2 at 0.5, 1, and 5 ppm in air
3. SO_2 at 0.5, 1, and 5 ppm mixed with NaCl aerosol
4. SO_2 at 0.5, 1, and 5 ppm mixed with distilled H_2O aerosol.

Before each challenge, the subject's vital capacity was measured by spirometry and the mean maximum expiratory flow rate at one-half vital capacity (MEF_{50} percent VC) was obtained. SO_2 nebulizer concentrations were constantly monitored by an automatic SO_2 analyzer. Each series of challenges was preceded by a 15-minute control period, during which the subject breathed ambient air from the apparatus. SO_2 was constantly monitored by an automatic SO_2 analyzer.

Five volunteers (including three from the previous experiment) participated in a second study designed to evaluate differences in effect between mouth and nasal inhalation of certain gas mixtures. Each volunteer breathed three types of gas samples through either a mouthpiece or a nasal mask. These samples included:

1. 5 ppm SO_2 in air
2. 0.5 ppm SO_2 in distilled H_2O aerosol
3. 5 ppm SO_2 in distilled H_2O aerosol.

MEF_{50} percent VC values were obtained in the same manner as in the first series, and measurements of total pulmonary resistance at functional residual capacity (TPR_{FRC}) were obtained at 4-minute intervals during each control and challenge period by the "oscillation technique."

Results from the first series of experiments showed that saline aerosol alone produced no significant change in MEF_{50} percent VC compared to control values. Distilled H_2O aerosol alone produced a small but statistically significant increase ($p < 0.05$) in flow rates after exposure. The authors attributed the differences in effects to particle mist size. Exposures to SO_2 at 0.5 ppm produced no statistically significant changes in MEF_{50} percent VC compared to controls, while 1 and 5 ppm SO_2 produced statistically significant decreases ($p < 0.02$ and $p < 0.01$ respectively) in MEF_{50} percent VC. Although no data were presented, the authors stated that a dose-response relationship existed.

For the test with SO_2 at 0.5, 1, and 5 ppm in combination with normal saline aerosol, no data were given, although the authors stated that all three levels of SO_2 delivered in NaCl mist produced a decline in MEF_{50} percent VC, but that only the results at 5 ppm SO_2 were statistically significant ($p < 0.01$). Subjects exposed to SO_2 with distilled H_2O mist experienced statistically significant decreases in these values from controls at all SO_2 levels ($p < 0.01$), but these decreases did not vary with the concentration of SO_2. Analysis of the particle sizes for the two aerosols revealed that the distilled H_2O mist particle size was in the submicrometer range, whereas saline aerosol generation produced particle sizes of 6.0 to 8.0 m at a higher particle concentration -- a condition that would favor greater deposition in the upper respiratory tract. The authors concluded that the ability of the two aerosols to effect a change in the pulmonary system appeared to be more related to size differences of the aerosol particles rather than to the chemical reactivity of SO_2 within each type of aerosol.

For all three mixtures of the second series of experiments, the subjects' average MEF_{50} percent VC compared to controls was higher after exposure by nose than by mouth, i.e., flow resistance for mouth breathing exceeded that for nasal breathing. SO_2 at 5 ppm in air produced the greatest decrease (10 percent), whereas SO_2 at either 0.5 or 5 ppm with distilled H_2O aerosol produced slight differences (compared to control values) in flow rates following exposure by the two administration routes. However, none of these findings were statistically significant. Mouth inhalation of all three mixtures resulted in a similar decrease (from controls) in conductance of approximately 20 percent for the SO_2-H_2O mixtures and 25 percent for SO_2 in air. Nasal inhalation of 5 ppm SO_2 resulted in a similar drop. At 0.5 ppm SO_2 with distilled H_2O aerosol breathed by nose, there was an average increase in conductance, compared to controls. However, at 5 ppm SO_2 with distilled H_2O aerosol inhaled by nose, a consistent and sometimes marked increase in conductance was observed, with two subjects more than doubling their control values. Changes in conductance were generally present at the 4-minute (first) measurement and were only slightly increased at the 12-minute (final) measurement. Subjective reactions were few; most frequently noted was a burning sensation in the throat following SO_2-H_2O mist challenge; for two subjects, this lasted for more than 1 hour after the exposure period.

Analysis:

The data presented for particle size distributions of water and saline aerosols are subject to question. Assuming that the authors measured particle size distribution on the same day so that factors such as relative humidity were constant, particle sizes for both aerosols should have been in similar ranges. The size differences presented here suggest that optical coincidence may have occurred in the aerosol photometer, since the peaks of both water and saline were in the range of 3000 to 3500 particles at two distinctly different particle sizes. Another shortcoming of this study is the lack of statistical analysis for

particle size distribution, including the number of observations, means, and standard deviations. Distribution of particle sizes are obviously not normal (i.e., bell-shaped), but are skewed, and the authors did not present any analytic clarification. Without these data, the information presented on particle size distribution is questionable. As a result, the scientific validity of this study cannot be assessed.

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b. Tracheobronchial Clearance

• Wolff RK, Dolovich M, Eng P, Rossman CM, Newhouse MT: Sulfur dioxide and tracheobronchial clearance in man. Arch Environ Health 30:521-527, 1975

Review:

Wolff et al. studied the effects of SO_2 exposure on tracheobronchial clearance in humans by quantifying the pulmonary retention of an inhaled radioactive aerosol by subjects breathing either room air or SO_2 at 5 ppm.

Nine healthy nonsmoking adults (seven men, two women; mean age: 24 years) underwent three experiments each at 1-week intervals. In each experiment, subjects first inhaled a radioactively tagged aerosol; retention of this aerosol was then measured for up to 3 hours in control experiments in which subjects breathed air or in test experiments in which subjects were exposed to 5 ppm SO_2 after aerosol inhalation. Mucociliary clearance was then compared under these conditions for each subject individually and for the group as a whole using two-tailed paired t-tests at a significance level of 0.05.

The aerosol (mass median diameter, 3 m) was ultrasonically generated from a 0.025 percent solution of technetium Tc 99m albumin in normal saline. The aerosol was inhaled as a bolus using a technique that limits aerosol delivery to late inspiration, thereby resulting in its deposition in large airways. The subject breathed first from a spirometer, then at late inspiration he or she breathed a 50-mL puff of aerosol from the nebulizer. The subject then exhaled into a bag and continued breathing room air through a separate valve system until the next aerosol breath was taken. This process was repeated until approximately 20 breaths of aerosol were inhaled within 3 to 4 minutes. The volume of aerosol was adjusted for each subject to correspond to the physiologic dead space of the large upper airways of the lungs as predicted from his or her height. Therefore, in each experiment any given subject inhaled the same number of aerosol breaths, of the same volume, at the same flow rate, with aerosol injected into the breath at the same volume above the functional residual capacity (FRC) of the lungs.

In control studies, immediately after inhalation of the aerosol, subjects entered a plexiglas chamber where they breathed air by mouth (noses were occluded) while measurements of lung radioactivity were taken at specific intervals over 3 hours. These measurements were made from a 5.0- x 10.0-cm region (control zone) over the right lung, centered at the carina.

Sulfur dioxide was metered from a tank of compressed 1 percent SO_2 into the chamber through a series of small orifices in an Teflon tube placed just behind the intake fan. In SO_2 exposure experiments, all conditions were identical, except that SO_2 was admitted to the control chamber and maintained at 5 ppm.

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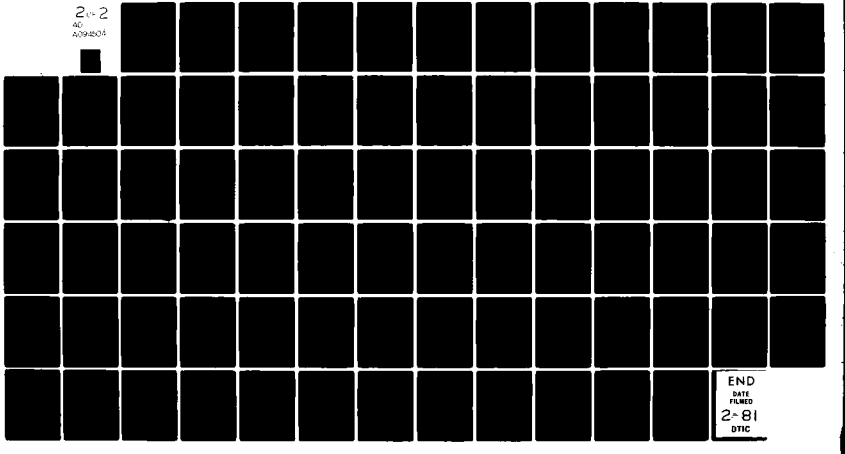

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Scintographs presented by the authors showed aerosol deposition to be predominantly in the large airways, as evidenced by the outlines of the main bronchi and trachea. This pattern of deposition was very similar in successive experiments for all nine subjects, and retention activity in relation to time was also similar for each of the nine subjects' two control studies for all times up to 2.5 hours past aerosol inhalation.

The effects of SO_2 on tracheobronchial clearance of 99Tc aerosol at 2 hours' postinhalation are presented in Table A-4. Individual values comparing aerosol retention in both control studies and in the 5-ppm SO_2 exposure study at 2 hours' postinhalation revealed no significant differences. One-way analysis of variance showed considerable variability from person to person for all three values ($p < 0.01$); however, the difference in retention for each person in all three measurements was very small ($p < 0.5$). A comparison of retention percentage change with time between the control studies and the SO_2 exposures showed that clearance was not markedly affected by SO_2 exposure, but actually increased slightly (about 8 percent) relative to control values at the 1-hour measurement ($p = 0.05$).

Spirometry was also performed after 2 hours' exposure, and Table A-5 shows a comparison of the results under control conditions compared to results during exposure to 5 ppm SO_2. No changes were found in vital capacity, forced expiratory volume at 1 second, or closing volume; however, there was a 10 percent reduction in maximal midexpiratory flow (MMEF) that was highly significant ($p < 0.01$). Because of the small sample size in this study ($n = 9$), the authors submitted the data to further calculations to determine if a significant difference in effect between the test conditions would have been more easily detectable if the sample size were larger. They calculated that there was a 2 percent chance of accepting a false hypothesis with a sample size of nine, and thus concluded that exposure to 5 ppm SO_2 does not markedly affect clearance in resting healthy adults, although there may be a transient change.

Measurements of tracheal clearance were possible in several instances when sufficiently discrete centers of activity moved up the trachea. The rates were similar for control studies and SO_2 exposures (1 to 6 mm per minute).

Subjective effects of SO_2 exposure included a slightly acrid, dusty taste, and a mild, throat-clearing cough lasting only the first 3 to 4 minutes of exposure.

The authors concluded that exposure to SO_2 at 5 ppm for 3 hours does not interfere with the essential pulmonary function of tracheobronchial clearance, and that, in fact, there is a small temporary increase in clearance as indicated by aerosol retention at 1 hour after inhalation and in MMEF at 2 hours after inhalation. They proposed that increased clearance may be due to stimulation of clearance mechanisms by the irritant response or by a change in mucus properties, ciliary activity, or both. An alternate explanation was offered that since SO_2

is highly soluble, most of it may have been absorbed in the mucosa of the mouth and pharynx and thus very little penetrated to the lung despite mouth breathing. Observed changes in MMEF were attributed to reflex bronchoconstriction.

Analysis:

The statistical procedures were thoroughly defined and appropriate for the data obtained from the study, showing a concern for the small sample size. The design and execution of the study are considered to be adequate and the results are therefore assessed as valid.

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TABLE A-4

Aerosol Retention at 2 Hours Postinhalation in Humans

Subject/ Sex/Age (yr)	Aerosol Retention, %		
	Control 1, Breathing Air	Control 2, Breathing Air	Breathing 5 ppm SO_2
1/M/23	73.0	68.0	73.0
2/M/25	63.0	69.5	67.0
3/F/23	60.0	53.5	63.5
4/M/24	28.5	35.5	22.0
5/F/21	77.0	80.0	69.0
6/M/23	59.0	52.0	50.0
7/M/24	70.0	63.0	66.5
8/M/27	75.0	84.0	73.5
9/M/25	64.0	65.5	68.0
Average ± SD	63.3 ± 14.6	63.4 ± 14.9	61.4 ± 16.3

Note: Table adapted from Wolff et al. Arch Environ Health 30:521-527, 1975

TABLE A-5

Human Pulmonary Function After 2 Hours[a]

Function	Air-exposed	SO_2-exposed	Difference	p value
Vital capacity (liters)	5.21	5.23	0.017 ± 0.031	0.2
$FEV_{1.0}$[b] (liters)	4.32	4.33	0.014 ± 0.053	0.95
MMEF[c] (liters/sec)	4.99	4.60	0.271 ± 0.09	0.01[d]
Closing volume (liters)	0.14	0.14	0.02 ± 0.02	0.8

[a] Figures represent mean values.
[b] $FEV_{1.0}$, forced expiratory volume at 1 second.
[c] MMEF, maximal midexpiratory flow.
[d] Significantly different.

Note: Table adapted from Wolff et al. Arch Environ Health 30:521-527, 1975.

c. Mode of Inhalation

• Speizer FE, Frank NR: The uptake and release of SO_2 by the human nose. Arch Environ Health 12:725-728, 1966

Review:

Speizer and Frank investigated the uptake and release of SO_2 by the human nose during quiet breathing.

Seven male volunteers (age range: 27-39 years) were fitted with masks through which they could breathe air or SO_2. Critical flow approximated 1 liter per minute. During the control period, subjects breathed only air; during the exposure period, each subject was instructed to initiate SO_2 exposure (delivered at approximately 25 ppm) immediately after the onset of either inspiration or expiration, and to continue exposure until just prior to the end of that phase of the breathing cycle. During SO_2 exposure (about 25-35 minutes) a spirometer recorded frequency of breathing and expiratory tidal volume. Inspiratory and expiratory air samples were collected from each subject at various points:

1. the respiratory tubing just outside the mask
2. within the mask, 2 cm in front of the nose and directly in the path of the airstream (mask-inspiratory - i)
3. within the nose 1 to 2 cm beyond the alae nasi (nose-i and nose-e)
4. within the oropharynx as far back as the subject could tolerate (pharynx-i and pharynx-e).

The SO_2 concentrations of these samples were measured using the electroconductivity method of Thomas and Abersold. Each subject was exposed a maximum of five times to SO_2; the interval between exposures was a minimum of 1 week.

Data collected during and after these SO_2 exposures are presented in Table A-6. At a point 1 to 2 cm within the nose (nose-i), the mean SO_2 concentration was 13.8 ppm; however, the mean SO_2 concentration in the inspired gas reaching the pharynx (pharynx-i) was 0.3 ppm, about 2% of the SO_2 concentration that had entered the nose. Expired gas in the pharynx (pharynx-e) also had a low level of SO_2, 0.4 ppm. However, during expiration through the nose, SO_2 was apparently released from the nasal mucosa, since the mean SO_2 concentration increased to 2.0 ppm. Data from the first and second recovery periods indicated that, although SO_2 concentrations were stable in the pharynx during inspiration and expiration, levels in the nose continued to decrease.

TABLE A-6

SO_2 Concentrations Outside of and Within the Upper Airways During Exposure and Recovery

Measurement Site	Mean SO_2 concentration (ppm)		
	During Exposure	1st Recovery Period[a]	2nd Recovery Period[b]
Inspiratory tubing	25.3	--[c]	--
Mask (i)[d]	16.1	--	--
Nose (i)	13.8	--	--
Pharynx (i)	0.3	0.3	0.2
Pharynx (e)[e]	0.4	0.4	0.4
Nose (e)	2.0	0.8	0.5

a First 15 minutes after exposure.
b Second 15 minutes after exposure.
c (--) indicates no data given.
d i = inspiration.
e e = expiration.

Note: Table adapted from Speizer and Frank. Arch Environ Health 12:725-728, 1966.

Analysis:

The design of this study was appropriate for the authors' objectives and the procedures were adequately executed. The statistical procedures used were not explained and therefore cannot be evaluated.

These results help confirm reports by other authors that the nose is extremely efficient in removing SO_2 from inspired air. In this study, approximately 2 percent of the concentration of SO_2 that entered the nose was detectable in the inspired air of the pharynx. In addition, in most subjects, the nasal mucosa released small amounts of SO_2 back into the expiratory airstream both during exposure and recovery periods.

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2. Environmental or Industrial Exposure

a. Accidental High-Level Exposure

The immediate and persistent effects of accidental human exposures to very high levels of SO_2 have been documented in several studies. However, in these cases, the SO_2 concentration producing those effects has not been measured or estimated; even exposure times are not always available. Although these case reports are of little value in helping to establish dose-response relationships, they do provide insights into the clinical symptoms and the respiratory tract pathology following high-level SO_2 exposure. The studies indicate that these exposures produce symptoms almost entirely related to the respiratory system. The symptoms are similar to those of lower-dose SO_2 exposures and include nose and throat irritation, eye irritation with copious lacrimation, and sneezing, and can also include a violent cough reflex that may act as a means of protection until the subject escapes from the exposure site. In cases of immediate fatality, postmortem changes are those of asphyxiation--inadequate pulmonary ventilation. When the symptoms do not appear for several hours and death is delayed, the changes are similar to those observed with the inhalation of any irritant, gas and death is most frequently caused by secondary pulmonary infection.

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• Rostoski O, Crecelius AP: Zur Kenntnis der Sulfitgasvergiftungen. Dtsch Arch Klin Med 168:107-122, 1930

Review:

Rostoski and Crecelius reported the acute SO_2 overexposure of 18 men in a factory, none of whom died immediately. However, two men later died, one at 10 months and another 15 months after the accident, both of secondary pulmonary infection. After 13 years, 8 of the 16 survivors remained incapacitated with bronchitis and/or emphysema (radiologically confirmed). Of the eight survivors who returned to work, all complained of shortness of breath (dyspnea) and bronchial catarrh.

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• Hamilton A, Hardy H: Industrial Toxicology, 3rd Ed. Publishing Sciences Group,Inc., Acton, Mass., pp. 209-211, 1974

Review:

Hamilton and Hardy reported the accidental SO_2 overexposure of 13 men in the semi-open hold of a ship, all of whom were rendered unconscious immediately. Fifteen minutes elapsed before the last man was

removed from SO_2 exposure. All the men experienced inflammation of the eyes, nausea, vomiting, abdominal pains, sore throat, and, later, bronchitis. One worker, who appeared to be recovering, died 10 days postexposure. Ten of the original thirteen men returned to work in 3 weeks apparently without subsequent symptoms. Two men continued to experience weakness and fever for several weeks, destruction of ciliated epithelium, and bacterial invasion of the lung. The authors did not report the order in which subjects were removed from the SO_2 exposure; thus it is not possible to correlate subjects' exposure times with symptoms and subsequent clinical sequelae.

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• Charan NB, Myers CG, Lakshminarayan S, Spencer TM: Pulmonary injuries associated with acute sulfur dioxide inhalation. Am Rev Respir Dis 119:555-560, 1979

Review:

Charan et al. reported pulmonary injuries associated with acute high-level SO_2 inhalation by five workers in a paper plant. Subjects 1 and 2 were exposed to SO_2 in an almost completely enclosed chamber and were assisted out by subject 3. Subjects 4 and 5, both wearing protective masks, were also exposed, but for a shorter period of time than subject 3.

Subjects 1 and 2 (ages 56 and 59 years, both nonsmokers) died of respiratory arrest within 5 minutesof initial exposure. The authors noted that these subjects had severe corneal opacification at death. Postmortem examination revealed that pharyngeal and laryngeal muscosa had a coagulated appearance. Microscopic examination showed extensive denudation of the superficial columnar epithelium, but with retention of basal cells. The lungs of both subjects were heavy, weighing more than 3000 g (normal weight is 600-800 g), and the airways were filled with pink edema fluid. Histologic examination of the undistended lungs revealed extensive sloughing and denudation of the mucosal surface of the large airways. Of note was the intact mucous glands and the absence of inflammatory cell infiltration. Similar observations were made in the small airways, some of which in addition were filled with a proteinaceous edema fluid. There was no disruption of alveolar walls; the alveolar sacs were filled with protein-rich edema fluid and, in some regions, extravasated blood. Acid hematin was noted, suggesting acidification of heme by the inhaled gas. Again, no inflammatory cells were noted. The sudden death of these subjects apparently allowed no time for inflammatory cell migration and infiltration, or fibrosis.

The authors reported that the cause of death in subjects 1 and 2 was probably a combination of extensive alveolar hemorrhage and edema, and the asphyxiating effects of the high concentration of SO_2 and

subsequent reflex vagal stimulation. The pulmonary edema in these subjects may have been due to both increased capillary permeability and increased capillary hydrostatic pressure. The mechanism of injury appeared to be direct SO_2-induced tissue damage.

Acute symptoms in the survivors (subjects 3, 4, and 5) were identical and included irritation and soreness of the nose and throat; tightness in the chest, and intense dyspnea; the eyes showed severe conjunctivitis and superficial corneal burns. The pharyngeal mucosa was hyperemic but without ulceration. These men had decreased breath sounds, diffuse rales and rhonchi, with essentially normal chest X-rays and no evidence of pulmonary edema. Arterial blood gases in subjects 4 and 5 revealed mild hypoxemia and hypocapnia easily corrected by low-flow oxygen. Subject 3 was more hypoxemic ($P_{a_{O_2}}$ of 72 mm Hg with P_{O_2} at 0.6 or 60%). Corticosteroids were prescribed. Within 1 week, the blood gases of subjects 4 and 5 returned to normal, while subject 3 continued to experience hypoxemia ($P_{a_{O_2}}$ of 65 mm Hg in room air). Pulmonary function tests (PFTs) revealed that on day 1 postexposure, subject 3 had mild airway obstruction, without an increase in lung volume; subsequent tests revealed severe, irreversible airway obstruction with air trapping and hyperinflation. Subject 4, with normal PFTs at day 1 postexposure, developed mild airway obstruction unresponsive to inhaled bronchodilators by day 116 postexposure. (Subject 4 was described as a 15 pack-year smoker). Compared to initial values, his FRC (functional residual capacity) and RV (residual volume) decreased slightly. Subject 5 had no pulmonary function abnormalities throughout the 116-day testing period. Subject 3 continued to experience dyspnea on mild exertion, despite vigorous bronchodilator therapy including steroid inhalation.

Of the survivors in this study, the two subjects with the longest SO_2 exposure (subjects 3 and 4; subject 4 wore a mask) had residual airway obstruction on recovery. The gradual deterioration in lung function as measured by spirometry suggested the obstruction was secondary to the exposure. The lack of deterioration in lung status in subject 5 was probably due to the protective mask and the minimal exposure encountered. The decreased FEF (forced expiratory flow) and FEV (forced expiratory volume) noted between days 1 and 116 postexposure in subjects 3 and 4 suggested gradual airway deterioration to the authors. The slightly decreased lung volume in subject 4 may have been due either to complete closure of terminal lung units secondary to bronchiolar fibrosis or to interstitial fibrosis following diffuse alveolar septal injury. The authors noted, however, the lack of previous reports in which SO_2 causes a restrictive abnormality.

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• Galea M: Case report: Fatal sulfur dioxide inhalation. Can Med Assoc J 91:345-347, 1964

Review:

Galea reported the accidental overexposure to SO_2, for 15 to 20 minutes, of two men working in a paper plant. Subject 1 was a 45-year-old male who smoked heavily. Pulmonary function tests performed 4 months postexposure revealed a satisfactory vital capacity (VC) of 86 percent of normal, a delayed chronometric VC, and prolonged expiration. In addition, the subject experienced hyperventilation at rest, a ventilatory equivalent of 39 liters (normal: 25-35 liters), which the author concluded to be indicative of marked respiratory fatigue. The subject had a maximum breathing capacity (MBC) reduced to 60 percent of normal, with improvement to 70 percent upon bronchodilator administration. While this subject experienced no dyspnea at rest, his capacity for exertion was markedly restricted.

Subject 2 was a 35-year-old nonsmoker previously in excellent health. Immediately after the SO_2 exposure, he complained (as did subject 1) of slight eye irritation and pain on deep breathing. Several days later he developed a dry, irritable cough, dyspnea, and copious amounts of mucus. He had rales at both lung bases, with highly audible wheezes. This subject deteriorated progressively, despite antibiotic, steroid, and bronchodilator therapy, and died 17 days after the SO_2 exposure.

In the autopsy findings, no pleural adhesions or effusions were present, but the lungs lacked elastic rebound. Thick mucus was present proximal to the trachael bifurcation. The gross impression was that of a diffuse type of acute emphysema. Histologic examination revealed extensive tracheobronchitis with ulceration, destruction of surface pseudostratified epithelium, and heavy inflammatory infiltration by polymorphonuclear cells. The inflammatory cell infiltration extended through the submucosa, beyond the hyaline cartilage rings of the trachea and into the fibroadipose tissue. Where the mucosa was preserved, a pseudomembranous exudate was present. The absence of edema and extravasated blood was probably due to its re-uptake into the tissues and various clearance mechanisms, e.g., pulmonary macrophages and mucus clearance, later followed by an intense inflammatory reaction resulting in residual scarring of lung tissue.

Another histopathologic feature was hypersecretion of mucus by the mucus-secreting acini of the submucosal glands of the trachea. Lesions were found in the terminal bronchioles and in some large airways. Plugging of airway lumina with an exudate of polymorphonuclear leukocytes was observed along with a striking proliferation of foamy macrophages. Examination of the terminal bronchioles revealed extensive replacement of the lamina propria by fibrosis, with encroachment on the lumen, no appreciable epithelial lining regeneration, and possible patchy metaplasia. Distension of the majority of alveoli and air sacs was also observed with rupture of alveolar septa. These septa were uniformly thin and generally avascular. The authors stated that the fundamental lesions

of extensive peribronchiolar fibrosis and bronchiolitis obliterans in this subject were presumably responsible for the acute emphysematous changes consistent with the cause of death.

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• Gordon J: Acute tracheobronchitis complicated by bronchial stenosis following the inhalation of sulfur dioxide. NY State J Med 43:1054-1056, 1943

Review:

Gordon reported the accidental SO_2 overexposure of an electrical repairman, aged 44, who had had a mild, upper respiratory tract infection 5 weeks before the SO_2 exposure. Before complete recovery from the infection, he was exposed to SO_2 for an unspecified number of minutes. Immediate symptoms included mild irritation of the nose and throat. Five days post-SO_2 exposure, he developed paroxysmal coughing and severe dyspnea, and he expectorated small amounts of tenacious, stringy sputum. Symptoms were not relieved by epinephrine. He was hospitalized in acute respiratory distress, with urgent dyspnea, orthopnea, and paroxysmal productive cough. Rales and rhonchi were present and X-rays revealed dense radiating strands in the upper third of the right lung, irregular, mottled shadows in the lower right lung and peritruncal thickening of the left lung. Fiberoptic bronchoscopic examination revealed a thin, grayish membrane covering the tracheobronchial tree and a detached, thick coagulum almost filling the lumen of each bronchus. Examination of expectorated fibrinous bronchial casts revealed purulent material with cell necrosis, many polymorphonuclear leukocytes, and large mononuclear cells; epithelial cells were not observed. Three months postexposure, he experienced occasional wheezing, slight dyspnea, and a moderately productive cough.

Five months after the first SO_2 exposure, this subject again inhaled SO_2 in the fumes of coke (for an unspecified duration of exposure). He was seized with a severe headache, chills, perspiration, vomiting, and a sense of chest constriction, followed by unconsciousness. After 1 week, he developed an acute infection of the upper respiratory tract and was hospitalized. Tactile fremitus was increased bilaterally and percussion was resonant and unimpaired. Breath sounds were bronchovesicular bilaterally and there were inspiratory and expiratory squeaks and palpable and audible rhonchi (especially over the left lung). X-rays revealed no striking abnormality and laboratory tests revealed only moderate leukocytosis. Bronchoscopic examination revealed that the left main bronchus was hyperemic and the lumen was reduced to three-fourths of its normal size. The stenosis of the left main bronchus was triangular in outline, moderately elastic and less than 7 mm in diameter. The subject's symptoms abated under conservative therapy and he was discharged about a month after the second SO_2 exposure.

b. Epidemiologic Data

• Kehoe RA, Machle WF, Kitzmiller K, LeBlanc TJ: On the effects of prolonged exposure to sulphur dioxide. J Ind Hyg 14(5):159-173, 1932

Review:

Kehoe conducted an epidemiologic study of workers exposed to relatively pure SO_2 gas arising from liquid SO_2 used as a refrigerant.

Subjects of the study included 100 men who had an average duration of work exposure of about 4 years, and who had been exposed during the work day to average concentrations of approximately 20-30 ppm (ranging from 5 to 70 ppm). An age-matched control group of 100 workmen was selected from other parts of the plant where no exposure to SO_2 or other potentially noxious agents such as paints, lacquers, and metallic dusts occurred. Subjects in both groups were questioned in detail about the length and nature of their occupational exposures and about symptoms associated with the exposures. Physical examination, complete blood counts, urinalysis, and chest X-rays were conducted on exposed and control subjects.

The symptoms associated with exposure were classified as: (1) initial symptoms (those that developed during the period of exposure before any acclimatization); (2) symptoms arising from minimum or customary exposure with or without acclimatization; and (3) symptoms produced by heavy exposure (i.e., irregular exposure to high concentrations). Initial symptoms were confined to the respiratory tract and, in descending order of frequency, consisted of irritation of the upper respiratory tract, coughing, epistaxis, constriction of the chest, and hemoptysis. Symptoms associated with minimal or customary exposures generally were milder forms of the initial symptoms, but were regarded as expressions of chronic, low-grade irritation. In descending order of frequency, these were hacking cough, morning cough, nasal irritation and discharge, prolongation of common colds, and expectoration. Symptoms arising from heavy exposures were generally intensified forms of those symptoms experienced during initial exposures and consisted of coughing, sneezing, conjunctival irritation, soreness of the throat and larynx, thirst, weakness, transitory chest pains, temporary loss of appetite, polyuria, and prolonged chest pain.

The Chi-square test was applied to the values obtained for physiologic findings in order to determine the level of significance. The authors reported a significant difference (confidence level not given) with respect to the average duration (but not frequency) of common colds in exposed subjects (13 weeks) as compared to that of control subjects (5.7 weeks). (It was not clear whether these figures pertained to weeks per cold or weeks of cold per year.) The frequency of dyspnea on exertion, fatigability, altered sense of taste and smell, and sensitivity to other irritants were positively and significantly ($p < 0.007$) associated with the frequency of severe exposures to SO_2. Clinical and laboratory findings that were found to be positively and significantly ($p < 0.007$)

associated with exposures included nasopharyngitis, tonsillitis, abnormal deep tendon reflexes, increased urine acidity, and lymphocytosis (Table A-7). There was no difference in the occurrence of chest X-ray abnormalities between the two groups.

The authors concluded that there was no appreciable danger to health from chronic exposure to "endurable" concentrations (20-30 ppm), and that even with frequent, intense exposures (up to 70 ppm), there were no serious or permanent health effects.

Analysis:

The statistical design of the study is considered appropriate, and statistical procedures were carried out accurately. However, the validity and usefulness of this study are limited by the techniques and equipment available in 1932. There is no way to assess the accuracy or sensitivity of the monitoring procedure used to detect levels of SO_2 in the plant during the exposure period. Also, had more modern and sophisticated diagnostic methods been available then, perhaps there would have been evidence of more serious health effects. These data must be viewed with extreme caution as preliminary evidence that the relatively brief, intermittent exposures that soldiers may experience are unlikely to result in any permanent health effects.

TABLE A-7

Effects of Long-Term Exposure to SO_2 in Industrial Workers

Significant Physical Finding[a]	Exposed Subjects (%)	Control Subjects (%)
Pharyngitis, chronic and slight	70	31
Chronic pharyngitis	38	9
Slight pharyngitis	32	22
Urine acid to methyl red	87	47
Urine alkaline to methyl red	10	53
Tonsillitis, chronic and slight	34	12
Abnormal reflexes	31	15

[a] Physical findings are arranged in decreasing order of statistical significance. All are significant at or beyond the $p = 0.007$ level.

Note: Table adapted from Kehoe et al. J Ind Hyg 14(5):159-173, 1932.

c. Skin Reactions

• Pirila V: Skin allergy to simple gaseous sulphur compounds. Acta Allergol 7:397-402, 1954

Review:

Pirila reported two cases of sulfur compound-induced skin reactions in humans.

The first subject was a 30-year-old male, exposed to sulfur compounds during employment in a sulfite cellulose factory. He suddenly developed headaches, itching, red patches, and wheals with white borders on his palms and, later, on other areas of the skin. The wheals developed only when he was working and disappeared almost totally when he was away from work for 2 days.

The second subject developed skin reactions initially in response to application of a sulfur-containing ointment, then after exposure to sulfur powder, and later when living near a factory emitting fumes resembling those of hydrogen sulfide.

For diagnostic purposes, the first subject received intracutaneous tests of 24 extracts including SO_2 (1:1,000,000; 1:100,000; 1:10,000; and 1:1000). Only the intracutaneous injection of SO_2 at the dilution of 1:1000 (administered as 0.01 ml) elicited a slightly positive response. Observations of his skin condition at his workplace, with and without a gas mask, made it apparent that SO_2 inhalation rather than skin contact caused the eruption. Clinical exposure tests included a 1-hour exposure to an unspecified concentration which resulted in small wheals on the subject's neck at 10 hours' postexposure. The subject was then challenged with 40 ppm SO_2 for 1 hour (the method of SO_2 determination was not described), 6 hours after which he developed urticarial wheals on the trunk and extremities. The eruption worsened during the following 9 hours, after which it gradually disappeared.

The author believed this subject's condition was SO_2-induced urticaria. The positive clinical exposure test in a chamber at 40 ppm SO_2 (0.004 volume percent) for 1 hour revealed that a relatively moderate exposure to SO_2 was sufficient to cause the urticarial wheals.

Diagnostic tests on the second subject with sulfur powder, carbon disulfide, and hydrogen sulfide were administered. Test results indicated that she had become sensitized to carbon disulfide and sulfur itself when exposed by skin contact, and to hydrogen sulfide when exposed in an inhalation chamber. This subject was presumably not exposed to SO_2 before treatment and diagnostic tests did not include SO_2.

Analysis:

These case studies are of general medical interest because they include descriptions of adverse skin reactions after exposure to SO_2 and other sulfur-containing compounds. They are of specific interest because of the data associated with exposure to 40 ppm for 1 hour by the first subject. It is of note that the skin reaction was delayed in onset and was totally reversible.

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• Pirila V, Kajanne H, Salo OP: Inhalation of sulfur dioxide as a cause of skin reaction resembling drug eruption. J Occup Med 5:443-445, 1963

Review:

Pirila reported the case of a skin reaction resembling drug eruption that resulted from SO_2 exposure. Neither the SO_2 concentrations nor the durations of exposure were known.

The subject was a 46-year-old male carpenter who was exposed on multiple occasions to high levels of SO_2 (concentration and duration of exposures unknown) while demolishing refrigerator machinery. On one occasion, after 3 consecutive days at this work, the subject observed an eruption on his forearms, which spread to his trunk and all extremities during the following 5 days. This eruption was accompanied by swelling of the eyelids. On physical examination, red papulae were observed all over the extremities, trunk, and face. On the groin, legs, and face, the papulae coalesced into red, infiltrated, scaling patches. Swelling of the eyelids was observed, with no signs of irritation of the conjunctivae or respiratory passages. Regression of the eruption began soon after the subject gave up his work and started topical and oral antihistamine treatment. The eruption resembled medicamentous exanthema (i.e., drug-induced skin eruption).

For diagnostic purposes, the subject was exposed to SO_2 in a gas-tight chamber. Exposure to 10 ppm SO_2 for 30 minutes was well tolerated and immediately after exposure no signs of irritation of the skin or mucous membranes were observed. On the following day, however, lesions similar to those observed on physical examination and lasting 1 day developed on the cubital fossae, along with a slight swelling of the eyelids and itching of the trunk. In a second diagnostic test, the subject was exposed to 40 ppm SO_2 for 10 minutes in the chamber, let out to breathe fresh air for an unreported period of time, then re-exposed to 40 ppm SO_2 for an additional 10 minutes (thus mimicking multiple, short, high-level exposures). Once again there was no immediate postexposure skin or mucous membrane reaction; only slight wheezing in the right upper thorax was revealed on lung auscultation. On the following day, a severe skin eruption developed in the cubital fossae, and spread all over the upper extremities. One day later the

eruption peaked; the papulae tended to coalesce and the eyelids became swollen. Regression of the lesions followed over the next few days.

Analysis:

Because neither SO_2 exposure levels nor their durations were available for the period of occupational exposure, the clinical findings reported for this subject are of limited usefulness. However, the results of the diagnostic tests do provide valuable data because they confirm the association of SO_2 with the occurrence of the skin eruption as well as give some quantitative parameters for those eruptions.

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B. ANIMAL STUDIES

1. Effects on the Respiratory System

• Ferin J, Leach LJ: The effect of SO_2 on lung clearance of TiO_2 particles in rats. Am Ind Hyg Assoc J 34(6):260-263, 1973.

Review:

Ferin and Leach studied the effect of multiple, intermittent SO_2 exposures on lung clearance of radioactive particles of titanium dioxide (TiO_2) in rats.

Groups of male rats were exposed to air or SO_2 gas in a dynamic flow chamber. SO_2 concentrations used were 0.1, 1, and 20 ppm for 7 hours per day, 5 days per week, for 10 or 25 days (thus total exposure times were 70 and 175 hours). The SO_2 concentration was monitored by a gas chromatograph with a flame photometric detector. For lung clearance determinations, an inert particle (TiO_2) challenge system was used. On the day following the last SO_2 inhalation, all animals were exposed to TiO_2 aerosol at a concentration of approximately 15 mg/m^3 for 7 hours. Both control and SO_2-exposed rats were sacrificed at 1, 8, 25, and approximately 136 days after the TiO_2 exposure. The total lung content of retained TiO_2 in individual rats was determined by photometry. Only the lung tissue, without the trachea, and without the mediastinal structures or lymph nodes, was analyzed. Data were presented as means and standard deviations. Student's t-test was used for statistical interpretation.

The initial lung burden (1 day after TiO_2 exposure) was approximately 150 g TiO_2 per rat. The retained amount of TiO_2 at 25 days after TiO_2 exposure averaged about 58 percent of the original lung burden, as determined from control groups of 20 different experiments (approximately 400 rats). The TiO_2 retention 25 days after TiO_2 exposure was often used as the control to which other clearance rates were compared.

As shown in Table A-8, in rats exposed to SO_2 at 0.1 ppm for 10 days or 23 days, the clearance of particles was higher than in the control groups (i.e., retention levels are lower in SO_2-exposed than in control rats).

In experiments with 1 ppm SO_2 concentrations, particle clearance was a function of the number of exposures to SO_2. Rats with 10 to 20 days of exposure to SO_2 had particle clearances at control or higher levels. Rats exposed to SO_2 for 25 days demonstrated depressed clearance of particles.

Rats exposed to 20 ppm SO_2 for 11 days showed a slight decrease in clearance compared to controls.

TABLE A-8

Effect of SO_2 Exposure on Lung Retention of TiO_2 in Rats 25 Days after TiO_2 Exposure

	Retention of TiO_2 (%)		
Days of Exposure	0.1 ppm SO_2	1 ppm SO_2	20 ppm SO_2
10	42	40	--[a]
Control	54	61	--
11	--	--	64
Control	--	--	56
18	--	58	--
Control	--	63	--
20	--	62	--
Control	--	63	--
23	47	--	--
Control	59	--	--
25	54	92	--
Control	48	61	--
Mean for all controls		58	

[a] Dash (--) indicates no measurements made.

Note: Table data from Ferin J, Leach LJ. Am Ind Hyg Assoc J 34(6):260-263, 1973.

Analysis:

The design and procedures used in this study were appropriate and adequately executed. However, the usefulness of these data is diminished because a single measurement (at 11 days of exposure) was obtained for the rats exposed to SO_2 at 20 ppm. The results of this study suggest that SO_2 exposure time has a marked effect on lung clearance. Short-term higher concentrations (20 ppm for 11 days) appeared to be better tolerated than longer lasting low-level exposures (1 ppm for 25 days). Another notable effect was the increased clearance of TiO_2 particles under certain circumstances, suggesting an initial beneficial or adaptive response to low levels of SO_2 exposure.

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• Spiegelman R, Hanson GD, Lazarus A, Bennett RJ, Lippmann M, Albert RE: Effect of acute sulfur dioxide exposure on bronchial clearance in the donkey. Arch Environ Health 17:321-326, 1968

Review:

Spiegelman studied the effects of SO_2 inhalation on the bronchial clearance of radioactive monodisperse ferric oxide particles in three miniature donkeys exposed to SO_2 at 26 to 713 ppm for 30 minutes.

The donkeys inhaled SO_2 through tubes inserted 12 inches into each nasal passage, with the mouth and nasal passages further sealed. Concentrations of SO_2 delivered were monitored three times at 10-minute intervals. SO_2 was measured by determining the time required for a known flow rate of the gas mixture to decolorize a solution of iodine and potassium iodide containing starch. The method was calibrated by titration with a standardized sulfurous acid solution.

At termination of SO_2 exposure, nasal tubes were removed and a face mask was immediately positioned for a 5-minute inhalation of a radioactive aerosol consisting of 1.8 μm ferric oxide particles with technetium sulfide Tc 99m. The first chest radioactivity measurements were made within 3 minutes after the end of radioactive ferric oxide exposure. Scintillation detectors were placed on either side of the donkeys, and radioactivity measurements were made with the axis of the detectors at two chest positions, over the vertebral column at the insertion of the first rib, and also at the sixth rib. Eight to 12 clearance experiments were performed on each of the three donkeys during a 2-month period. Half of the clearance experiments were control studies (inhalation of radioactive ferric oxide only) performed on days preceding each SO_2 exposure.

In a control study, donkey 2, exposed only to ferric oxide particles, demonstrated a rapid and continuous clearance, ending in about 2 hours. When donkey 2 was exposed first to 300 ppm SO_2 for 30 minutes, then to the ferric oxide aerosol, there was a slow rate of lower lung clearance and a large wave of activity in the upper lung with a 1.5-hour lag in the onset of upper lung clearance. Control clearance for donkey 1 was similar to but more rapid than that of donkey 2. Clearance after 500 ppm SO_2 showed the same impairment of clearance as observed in donkey 2 after 300 ppm exposure. In both cases, clearance from the lower lung was abnormally slow and clearance from the upper lung was abnormally delayed, compared to controls. Donkey 1 exposed to 713 ppm showed even more severe effects: the clearance was markedly impaired for a period of 3.5 hours after which there was an abrupt resumption of rapid clearance. Donkey 3, exposed to 666 ppm SO_2, showed severe impairment of clearance that persisted to the following day.

Bronchial clearance was quantitated in terms of the weighted average time that particles remained on the bronchial tree. The method of calculation was the division of the area under the bronchial clearance curve (minus residual chest activity at the end of clearance periods) by

the total amount of radioactivity lost during bronchial clearance. No increase in the weighted average bronchial clearance time was observed for SO_2 exposures below 300 ppm.

The maximum effect on bronchial clearance was observed at SO_2 exposures ranging from 557 to 713 ppm, and was characterized by a sixfold increase in the average bronchial clearance time in the three donkeys. Recovery was reasonably complete even after exposures that produced marked impairment except in donkey 3, which had not recovered 1 day after exposure to 666 ppm of SO_2. In addition to impaired clearance, all three donkeys showed an abnormally large initial wave of activity in the upper lung field and a considerable lag in clearance onset. The increased duration and magnitude of the tracheobronchial wave with high-level SO_2 exposures probably reflected both the decreased rate of lower lung clearance and the slowing of mucus transport in the upper bronchi and trachea.

In the donkeys, absorption by the nasal passages was avoided by the use of the plastic catheters. However, SO_2 absorption probably occurred in the nasopharynx and along the 18-inch length of trachea. The donkeys exhibited discomfort during exposures to all concentrations of SO_2; however, impaired clearance occurred only when the SO_2 caused severe coughing and mucus discharge from the nose during exposure and persistent coughing during postexposure.

Analysis:

The authors failed to mention the advantage of using the miniature donkey as an animal model. It would seem that a larger number of animals would have provided more useful data. Statistical procedures were not applied in this experiment.

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• Frank NR, Speizer FE: SO_2 effects on the respiratory system in dogs. Arch Environ Health 11:624-634, 1965

Review:

Frank and Speizer studied the changes in functional response at different levels of the respiratory system in mongrel dogs during acute exposure to SO_2 at 7 to 61 ppm by nose and by tracheal cannula.

In the first set of experiments (nasal exposure), eight anesthetized, spontaneously breathing dogs were placed on their backs in a body plethysmograph with their mouths sealed to ensure nasal breathing. A latex mask was placed over the snout and made airtight. Pressure was measured at four sites: mask, pharynx, upper trachea, and pleural space. Pleural pressure was measured by a catheter inserted into the pleural cavity.

Respiratory rate (RR) and tidal volume (V_t) were measured with a 5-liter Krogh spirometer attached to the body plethysmograph. Volume was continuously monitored to provide the flow rate. The flow resistance of the nose (R_N) was measured by relating the pressure drop between the mask and the pharynx to the flow rate; flow resistance of the larynx (R_{LX}) was measured as the pressure difference between the pharynx and upper trachea total pulmonary resistance (R_L) was measured as the pressure difference between the upper trachea and pleural space. R_N and R_L were recorded simultaneously, followed within one to two breaths by the recording of R_{LX}. All values represented an average of inspiratory and expiratory flow resistance at isovolumes in the midtidal range for five consecutive breaths. Measurements of pulmonary compliance (C) were based on 10 breaths. The end-expiratory gas volume of the entire respiratory system, functional residual capacity (FRC), was measured by a method that relates changes in thoracic gas volume and airway pressure during obstructed breathing. Corrections were made for the volume of the mask and connecting tubes within the plethysmograph. The concentration of SO_2 leaving the mixing chamber was monitored by the electroconductivity method of Thomas and Abersold.

Two of four sets of control measurements were taken before SO_2 exposure. Measurements were then repeated during and after continuous SO_2 exposures at 2.5, 5, 10, and 20 minutes. Usually SO_2 exposure was discontinued after 20 minutes, except in a few cases where it was prolonged for 30 to 60 minutes. Measurements were also begun 5 minutes postexposure to SO_2. Most of the dogs were re-exposed to SO_2 20 minutes after the end of the previous exposure period and the values obtained during the postexposure or recovery period were used as controls for the subsequent exposures.

Following each completed set of experiments per dog, the dog was sacrificed and the lungs were excised, weighed, and examined for gross evidence of edema.

Airflow resistance was reported in terms of total lung resistance, i.e., $R_{total} = R_N + R_L$. Data for the eight control dogs spontaneously breathing ambient air indicated that the total flow resistance, R_N, comprised 65.6 percent (range: 48-82 percent), R_{LX} comprised 10.8 percent (range: 5-17 percent), and R_L comprised 23.6 percent (range: 11-39 percent) for the mean values of the eight control dogs. The sum of the inspiratory and expiratory flow rates at which these measurements were made averaged 0.78 liters per second (range: 0.60-1.09 liters per second). There was little difference between inspiratory and expiratory R_N for all observed rates of flow.

Eight experimental dogs were then exposed to SO_2 at concentrations ranging from 7 to 61 ppm for 20 minutes. The SO_2 concentrations were divided into three groups: low (7-16 ppm), medium (25-34 ppm), and high (60-61 ppm). R_N for 7 to 16 ppm SO_2 increased in a manner approximately proportional to the level of SO_2 administered: at the two higher levels of SO_2, the change in R_N became greater with the longer duration of exposure as well as in relation to the SO_2 concentration. For SO_2 levels greater than 16 ppm, R_N appeared to be proportional to time multiplied by concentration (Ct). Chi-square (x^2) analysis was used to test for significant differences between exposure groups. The significance level was set at $p < 0.01$.

During the exposures, R_L measurements rose about as often as they fell in a manner unrelated to either concentration or duration of exposure. Compared to control R_L levels, less than one-third of all 69 measurements taken during the 20-minute exposure proved significant (p 0.05). During recovery, R_L tended to increase to above control levels whether all changes or only statistically significant changes (at $p <$ 0.01) were considered.

R_{LX} also tended to rise and fall about an equal number of times during exposure to and recovery from SO_2.

Pulmonary compliance during exposure was decreased by about 5 percent for the entire group during both control and recovery periods. Although the difference was significant ($p < 0.01$), the authors questioned its physiologic significance.

Minute ventilation fell an average of 0.3 liters per minute during exposure ($p < 0.01$), due to slight reductions in both tidal volume (V_t) and respiratory rate (RR). There were no consistent trends in end-expiratory volume or in end-expiratory transpulmonary pressure during SO_2 exposure. A transient shift in lung volume, approximately 20 to 25 percent of control values, occurred infrequently in dogs exposed to intermediate and high SO_2 concentrations. Neither the changes in lung volume nor transpulmonary pressure correlated significantly with changes in R_L.

In the second phase of the study, Frank and Speizer studied the effects of SO_2 administered to seven dogs (dogs 9 to 15) by tracheal cannula inserted near the upper and middle two-thirds of the tracheal wall. Two of the dogs were paralyzed with the neuromuscular depolarizing agent, succinylcholine, and were ventilated with a Starling pump. (It is

assumed that the remaining five dogs were anesthetized but breathing spontaneously.)

Resistance to airflow in the larynx (R_{LX}) in the initial control period averaged 4.4 cm H_2O per liter per second (range: 1.6-7.6) compared with only 2.2 cm H_2O per liter per second (range: 1.2-4.3) for the group that had intact airways and breathed by nose (during the initial control period). End-expiratory transpulmonary pressure averaged 4.8 cm H_2O (range: 3.6-6.9) in nasal-breathing animals compared with only 3.4 cm H_2O (range: 2.7-4.0) in tracheostomized dogs.

In experiments with four dogs, SO_2 was administered directly to the trachea and lungs at concentrations of 60 to 215 ppm for 20 minutes. Despite the few data and the variability of response, the pattern of change of R_L with respect to time was similar to the results in which the isolated tracheolaryngeal segment was directly exposed to SO_2. The bronchoconstriction was most pronounced during the first few minutes of SO_2 exposure, then tapered off during the exposure period. However, the magnitude of the change in bronchoconstrictive tone was greatest when the lungs were directly exposed. However, R_L occasionally fell below control levels during both types of exposures to SO_2, i.e., exposures to the upper or lower segments.

Autopsy revealed that the lungs of dog 5 were extensively infiltrated with granulomata and contained secretions in many of the airways, and dogs 8 and 13 had "foam" in lower lobes of the lungs. However, none of these dogs demonstrated an exaggerated response to SO_2 administration.

The authors concluded that changes in R_N in this study probably reflected mucosal swelling and/or increased secretions. They offered no specific reasons for the weak response of the lower airways to SO_2 exposure to the nose, for the fluctuating response (i.e., the widening and narrowing of airways), or for the occasional delay in airway narrowing until the termination of SO_2 exposure. These changes could not be accounted for by shifts in lung volume. However, three possible explanations for their results were entertained. First is the great percentage of SO_2 uptake by the nasal passages, thereby greatly reducing the amount of SO_2 entering the lower airways. When higher concentrations were given by tracheal cannula, thus bypassing the nose, R_L increased dramatically, presumably due to the higher SO_2 levels reaching the lower airways. However, the bronchodilation that occasionally occurred with nasal SO_2 exposure could not be accounted for by such an explanation.

The second possible explanation for the observed phenomena involves the mechanisms by which SO_2 may induce changes in airway caliber. In this study, SO_2 administered by tracheal cannula induced R_L increases extremely rapidly with fluctuations not compatible with mucosal swelling or intraluminal edema. Further, at autopsy, most of the lungs appeared normal. The possibilities of SO_2 causing release of a histamine-like substance, acting directly upn tracheobronchial smooth muscle, or inducing intramural reflexes were mentioned. Since little or no SO_2

appears to reach the lower airways, the authors concluded that the precise location of the receptor tissue (or tissues) remains uncertain. The responses to SO_2 may involve not only direct action of the gas on smooth muscle and reflex action by the vagosympathetic nervous system, but possibly a variety of local, reflex, and humoral controls, including fear and apprehension of the unanesthetized subject.

Analysis:

The statistical design and procedures of this study appear to be adequate and the results are accepted as scientifically valid. It should be noted, however, that the test animals were kept under sodium thiopental anesthesia, their mouths were sealed, and a plaster mask, overlaid with latex, was placed over the snout and sealed to the head. The extreme physical manipulation of these animals may have influenced the nature of the reported results as compared to control animals spontaneously breath- ing ambient air.

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• Weiss KD, Weiss HS: Increased lung compliance in mice exposed to sulfur dioxide. Res Commun Chem Pathol Pharmacol 13(1):133-136, 1976

Review:

Weiss and Weiss studied the effects of SO_2 on lung compliance and other parameters in mice.

Fifty adult female mice (25 g average weight) were continuously exposed to SO_2 at 40 ppm for 6 to 9 days in a flow-through chamber. SO_2 was delivered from a compressed-gas tank set to deliver 8 ml/min by rotameter. The calculated SO_2 concentration was confirmed within $^{+}15$ percent by Bendix Gastec tubes using a Kitagawa hand pump. Food and water were given ad libitum except in two trials in which the controls were match-fed to the previous days' intake of the exposed mice.

Pulmonary compliance (C) and alveolar stability were measured from static volume-pressure (V-P) curves from the lung in situ. In some mice, after air V-P curves were established, the lungs were excised, weighed both submerged and in air, dried for 24 hours at 105° C, and reweighed. In these lungs, edema was evaluated from wet weight divided by dry weight. Trapped gas was measured by lung buoyancy. In other mice, a saline V-P curve was determined after air inflation and used to estimate the contribution of lung tissue and surrounding surface forces (e.g., chest wall) to lung compliance.

Exposed mice lost 21 percent of body weight compared to their pre-exposure weights (reported as significant, confidence level not stated). The 10 control mice fed ad libitum gained weight, but the 14 matched-fed controls lost weight at approximately the same rate as the SO_2-exposed mice. However, pulmonary function values of the controls were not affected by the reduction in feed intake. Lung compliance (from air V-P curves) was significantly increased by 37 percent for 21 SO_2-exposed mice compared to the 20 control mice ($p < 0.01$). No other statistically significant changes occurred in pulmonary status. The authors found an increase of 8 percent in tissue compliance (by the saline V-P curves) and of 17 percent for trapped gas in SO_2-exposed mice as compared to controls. Alveolar stability, lung weight, and the lung wet weight/dry weight ratio registered small decreases of 2, 2, and 6 percent, respectively.

The authors stated that the significantly increased pulmonary compliance of the exposed mice was probably not an artifact of weight loss, since the compliance of the matched-fed controls was normal. The increased compliance was compatible with the absence of edema. It appeared to be caused primarily by a decrease in surface tension rather than tissue elasticity, since the slopes of the saline V-P curves were only 8 percent greater when compared to control group. The authors believed that since alveolar stability was unchanged, neither surfactant concentration nor the geometry of the individual alveoli was greatly altered; however, the nonsignificant 17 percent increase in trapped gas may have indicated the recruitment of normally closed alveoli.

Analysis:

A major limitation of this study is that the authors used a range of exposure time (6-9 days) rather than a specific number of days. Thus some mice were exposed for up to 50 percent longer than others. This point was never addressed by the authors and data for all exposed mice were presented together. Other parameters--such as surfactant-surface tension--that may have supplied more useful information were not measured. The study, therefore, is of limited value.

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• Asmundsson T, Kilburn KH, McKenzie WN: Injury and metaplasia of airway cells due to SO_2. Lab Invest 29(1):41-53, 1973

Review:

Asmundsson et al. performed an extensive series of experiments on rats and hamsters to determine the morphologic changes produced by repeated injury to airway epithelial cells by SO_2 at 40 to 400 ppm, the time course of these changes, their resemblance to goblet cell hyperplasia and squamous cell metaplasia, the synergistic effects of SO_2 and carbon dust combined, and whether a model of bronchitis that is not due to infection can be constructed.

Young male albino rats and young male Syrian hamsters were used for the three groups of experiments:

1. Exposure to various SO_2 concentrations for 5 hours per day, 5 days per week

2. Exposure to various concentrations of SO_2 for one 4-hour exposure period

3. Exposure to 40 ppm SO_2 and carbon dust (0.74 g/m^3) for one 4-hour period.

Individual protocols and reported results are given in Table A-9.

In an attempt to duplicate the Reid model of chronic bronchitis, 30 rats were exposed to 400 ppm SO_2 for 5 hours per day, 5 days per week for 21 exposure days. The concentration of SO_2 in the chambers was monitored by a modification of the hydrogen peroxide acid titration method. Three rats died at the end of the first 5-hour exposure and a total of 22 rats died during the first week. The remaining rats appeared to tolerate the gas better but remained inactive. Three additional rats died between days 12 and 18 of the exposure.

Gross examination of both control and experimental animals revealed extensive degeneration of tracheal epithelia, leukocyte infiltration, and bacterial infection of the trachea and lungs. The authors thus chose to disregard these results and to continue the series of experiments with Syrian hamsters, a species known to have a low incidence of spontaneous respiratory infection.

Upon exposure to 400 ppm SO_2 for 5 hours per day, each of 6 hamsters immediately began to develop respiratory distress, with one animal dying within the first 45 minutes of exposure and the longest survivor dying on day 2 after a total exposure of 6.3 hours. Pathologic changes were similar to those found in the rats, with marked vacuolar degeneration of tracheal epithelium and large areas of necrosis and hemorrhage. Effects were diminished in more distal airways, with bronchi showing less extensive damage and some bronchioles showing some patchy vacuolation and degeneration of cells.

Table A-9

Experimental Protocols and Results of Exposure to SO_2 by Rats and Hamsters

	Species	No. of Animals	Conditions of SO_2 Exposure	Objective	Results
1.	Rats	30	SO_2 400 ppm 5 hr/day, 5 day/wk for 23 days (17 exposure days)	To duplicate the Reid model of chronic bronchitis (18)	Exposed rats died rapidly - 3 within 5 hr, 22 within 5 days. Remaining rats seemed to be more tolerant. Control and exposed animals developed pulmonary infections. Results considered suspect.
2.	Rats	6	Filtered room air	Controls	
3.	Hamsters	6	SO_2 400 ppm 5 hr/day, 5 day/wk	To assess survival and histologic changes in tracheobronchial tree	All animals died within 4 min to 6.3 hr of exposure.
4.	Hamsters	6	SO_2 40 ppm 5 hr/day, 5 day/wk for 6 wk (30 exposure days)	To see whether tolerance could be developed against SO_2 by gradual increases in dose. To assess histologic changes in tracheobronchial tree, including goblet cell metaphasia on long term exposure, to see whether diseases in the tracheobronchial tree correspond in severity to increasing concentrations of SO_2	Some loss of cilia and vacuolization of ciliated tracheal cells
5.	Hamsters	6	SO_2 100 ppm 5 hr/day, 5 day/wk for 6 wk (30 exposure days)		More extensive degeneration of ciliated tracheal cells
6.	Hamsters	6	SO_2 200 ppm 5 hr/day, 5 day/wk for 6 wk (30 exposure days)		Loss of most tracheal cilia, vacuolization, and extrusion of tracheal ciliated cells within 5 hours. Extensive ultrastructural changes detected by electron microscopy.
7.	Hamsters	7	SO_2 250, 330, 350, and subsequently 400 ppm, 5 hr/day, 5 day/wk for 7 wk (35 exposure days)		Changes were similar to, but more extensive than, those seen with exposure to SO_2 at 200 ppm. Basal cell hyperplasia, squamous cell metaplasia, and cell necrosis were extensive.
8.	Hamsters	8	SO_2 40 ppm 5 hr/day, 5 day/wk for 8 days (6 exposure days)	To study the time sequence of changes caused by SO_2 with the airways	Loss of cilia, vacuolization, cell extrusion, basal cell hyperplasia, and transitional cell metaplasia were observed later at 40 ppm than at 200 ppm.
9.	Hamsters	4	SO_2 200 ppm 5 hr/day, 5 day/wk for 8 days (6 exposure days)		
10.	Hamsters	8	SO_2 40 ppm for 4 hr	To study leukocyte recruitment in airways and the time sequence of the response after acute exposure and to determine the possible synergism between SO_2 and carbon particles	Exposure to combined SO_2 and carbon dust (but not to SO_2 alone) resulted in leukocyte infiltration of the lumens and bronchial walls within 24 hr. The results of exposure to carbon dust alone were not reported.
11.	Hamsters	12	SO_2 40 ppm and carbon dust, 4 hr		
12.	Hamsters	8	Carbon dust (0.74 g/m^3) 4 hr		
13.	Hamsters	43	Filtered room air	Control	

Note: Data from Asmundsson *et al.* Lab Invest 29(1): 41-53, 1973.

• Frank NR, Yoder RE, Yokoyama E, Speizer FE: The diffusion of $^{35}SO_2$ from tissue fluids into the lungs following exposure of dogs to $^{35}SO_2$. Health Phys 13:31-38, 1967

Review:

Frank et al. measured the expiration of $^{35}SO_2$ by mongrel dogs whose upper airways (nose, pharynx, larnyx, and upper trachea) had been surgically isolated and exposed to $^{35}SO_2$.

$^{35}SO_2$ was delivered at about 22 ppm by mask to the nasal passages of six anesthetized dogs for 30 to 60 minutes. In a second series of tests, four dogs (1, 2, 4, and 5) were exposed to 22± 2 ppm $^{35}SO_2$ for 30 to 38 minutes and one dog (3) was exposed to 22± 2 ppm $^{35}SO_2$ for 60 minutes. ^{35}S in expired gas was sampled from two catheters placed at the carina and 5 cm into the right main stem bronchus. SO_2 was measured conductometrically by means of a vibrating reed electrometer.

As shown in Table A-12, measurements taken for five dogs displayed an uptake (rate of absorption) of at least 99 percent of the $^{35}SO_2$ administered during the first 20 minutes of measurement. After 25 minutes, uptake values began to decrease somewhat, suggesting a point of blood or tissue saturation.

During $^{35}SO_2$ exposure of the isolated upper airways, ^{35}S appeared in expired gas from the bronchial and carinal levels in at least one sampling period for each animal. Concentrations were variable and no consistent differences were observed in ^{35}S between the carina and the bronchus in relation to concentrations or time. Gas samples drawn from the same sites every 20 to 30 minutes postexposure yielded similar results. Gas containing ^{35}S was detectable in the expired air of dog 1 for as long as 60 minutes postexposure.

In another series of tests, blood samples were drawn from the aorta through the right femoral artery at 5- to 10-minute intervals for dogs 1, 2, 3, 5, and 6. Samples were obtained both during and after upper airway exposure of 22 ± 2 ppm $^{35}SO_2$ for 30 to 38 minutes for four dogs and for 60 minutes for dog 3. Blood samples were also obtained from the external jugular vein draining the exposed area of the head.

The appearance and distribution of ^{35}S in the whole blood were stated as similar for all animals tested, although methods and data were not reported. ^{35}S appeared in the first sample of arterial whole blood taken during exposure, i.e., at 5 minutes. The level of radioactivity increased throughout exposure, became maximal near or at the end of exposure, and showed no evidence of blood saturation in any of the experiments, including the 60-minute $^{35}SO_2$ exposure. The arterial whole blood radioactivity declined slowly in the 1- to 2-hour postexposure period; experiments were not extended beyond this period.

The next series of experiments was designed to determine if tolerance to SO_2 could be developed by exposure at gradually increasing concentrations, and to determine if goblet cell changes and disease in the tracheobronchial tree corresponded in severity to increased SO_2 concentrations. Sections from tracheas, bronchi, and bronchioles were examined by light and electron microscope. Goblet cells were microscopically identified in tracheas and bronchi (1) as PAS-stained apical granules in sagital tissue sections (basal cells excluded) and (2) as cells without cilia containing clear apical vacuoles in tissue cross sections, using Richardson's stain. Goblet cells were counted as percent of total cells touching the lumen. Data represented an average of three or more tracheas, four or more bronchi, and four or more bronchioles in three or more animals. Differences in means were compared for significance using the Student's t-test. The experimental animals were exposed to 40 ppm (six hamsters), 100 ppm (six hamsters), and 200 ppm (six hamsters). A fourth group of seven hamsters was exposed to SO_2 at an initial concentration of 250 ppm that was gradually increased over a 4-day period to 400 ppm. The groups were exposed to SO_2 5 hours per day, 5 days per week for 30, 30, 30, and 35 total exposure days, respectively. These levels of SO_2 produced epithelial damage in large airways of all groups after 25 hours of SO_2 exposure. Changes became more severe and extensive with higher SO_2 concentrations and longer exposures. Even exposure to 40 ppm SO_2 for 1 to 6 weeks produced some loss of cilia and vacuolation of ciliated tracheal cells, which became more extensive during weeks 3 to 6 of the experiment.

Exposure to 100 ppm SO_2 for 30 days produced tracheal changes including extensive loss of cilia, degeneration of ciliated cells, and areas of basal cell metaplasia. Five of six hamsters showed nonextensive bronchial changes, with bronchioles appearing normal for all six hamsters. The ratio of goblet cells to total airway cells was unaltered in tracheas or bronchi and weight gain was normal.

A single 5-hour exposure to 200 ppm SO_2 resulted in the loss of almost all tracheal cilia. Most cells were vacuolated and appeared to be in the process of extrusion. Tracheal goblet cell numbers and weight gain were significantly decreased in comparison to the controls ($p < 0.0005$ and $p < 0.05$, respectively). Electron microscopy revealed that ciliated and goblet cells were larger than those of controls, and intercellular spaces were wide and vacuolated. The endoplasmic reticulum, nuclei, and mitochondria were extensively altered. Control hamsters had normal tracheal morphology. After 4 days (20 total hours) of exposure to 200 ppm SO_2, basal cell metaplasia was extensive in tracheas of five of the six hamsters. Goblet cell metaplasia was not observed. Bronchi showed loss of cilia and cellular degeneration, but neither basal cell nor squamous hyperplasia. Bronchioles appeared normal. The number of tracheal goblet cells was significantly reduced; however, the number of goblet cells in the bronchi was normal.

In hamsters exposed to 400 ppm SO_2 by gradual increments from 250 ppm over a 4-day period, after 1 week many ciliated cells were being extruded, but the percentage of goblet cells among tracheal cells was

comparable to control values. After 4 weeks, there were areas of cellular necrosis, basal cell hyperplasia, and squamous cell metaplasia. Changes in bronchi were similar to those in hamsters exposed to 200 ppm SO_2; bronchioles remained intact. No pathogenic bacteria or mycoplasma were found in any group of hamsters. After 3 to 6 weeks of exposure, goblet cell counts in tracheas and bronchi were not increased in any group, while weight gain was one-half that of controls.

The authors also examined the time course of changes due to exposures of 40 and 200 ppm SO_2 for 5 hours per day, 5 days per week, for a total of 6 exposure days. Ciliary loss was observed after 1 day of exposure to either concentration. Vacuolation and cell extrusion were also seen after 1 day at 200 ppm, and after 2 days at 40 ppm. Basal cell hyperplasia was later observed, and after 4 days at 200 ppm SO_2, small areas of transitional cell metaplasia were observed. Neither 40 nor 200 ppm SO_2 produced leukocyte infiltration of the trachea (a phenomenon that occurred at 400 ppm SO_2 within a few hours).

In order to investigate a possible synergistic effect from exposure to a combination of SO_2 and carbon dust, eight hamsters were exposed to 40 ppm SO_2 alone for 4 hours, twelve hamsters to 40 ppm SO_2 with carbon dust for 4 hours and eight hamsters to carbon dust alone (0.74 g/m^3) for 4 hours. At 12-, 24-, and 44-hour measurements, the number of polymorphonuclear leukocytes per segmental bronchus was similar to control values. When hamsters were exposed to 40 ppm SO_2 and 0.74 g/m^3 of carbon dust combined, numerous leukocytes were observed in bronchial walls and lumens 24 hours after the start of the exposure.

Unpatterned, occasional positive bacterial cultures appeared in both control and exposed hamsters. Microscopic examination revealed no bacteria or mycoplasma, suggesting that infection had no role in damage or altered differentiation of airway epithelium in hamsters exposed to SO_2.

The authors reported the following sequence of airway epithelial changes in hamsters in response to nonfatal SO_2 exposures: first, dilation, vacuolation, extrusion, and exfoliation of ciliated and goblet cells occurred in a few hours, followed after 3 to 4 days of continuing SO_2 exposures, by replacement of ciliated cells by goblet cells. By days 6 to 8, the goblet cells were lost, replaced by areas of basal cell hyperplasia and transitional metaplasia. The final change after 2 to 4 weeks of exposure to SO_2 above 200 ppm was squamous metaplasia.

Analysis:

The authors did not report the results of polymorphonuclear leukocyte infiltration for the eight hamsters exposed to carbon dust alone for 4 hours; thus, interpretation of the experiment is difficult because the carbon dust alone may have been responsible for the leukocyte recruitment observed in the 12 hamsters exposed to both SO_2 and carbon dust.

The results of these studies provide a valuable assessment of the microscopic and ultrastructural changes that can be expected to occur in the trachea after subchronic exposure (6 weeks) to high levels of SO_2.

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2. Absorption and Distribution

• Frank NR, Yoder RE, Brain SD, Yokoyama E: SO_2 (^{35}S labeled) absorption by the nose and mouth under conditions of varying concentration and flow. Arch Environ Health 18:315-322, 1969

Review:

Frank et al. studied the absorption of $^{35}SO_2$ at 1 to 50 ppm by nose and by mouth in dogs, in order to determine whether SO_2 concentration or the ventilatory flow rate was the more important factor in the degree of airway penetration by SO_2 and the subsequent response of the lower airways.

The authors anesthetized and paralyzed seven adult dogs, and infused them with life-supportive fluids. Tracheal cannulas were inserted, isolating the upper from the lower airways, and the animals' lungs were ventilated with ambient air. The dogs were fitted with masks through which compressed air or SO_2 could be directed into the isolated airways through either the nose or mouth. The $^{35}SO_2$ leaving the upper airway segment was monitored continuously during and after each experiment conductometrically by an ion chamber coupled to a vibrating reed electrometer. The concentration of $^{35}SO_2$ administered to the isolated upper airway ranged from 1 to 50 ppm for approximately 5 minutes. Two flow rates were used: 3.5 and 35 liters per minute. At 35 liters per minute, the periods of exposure were usually shorter due to rapidly increasing penetration of the airways by $^{35}SO_2$, which reduced the sensitivity of the monitoring electrometer to additional exposures on the same animal.

Desorption of $^{35}SO_2$ from the isolated upper airways following exposure was also investigated by the authors. Following $^{35}SO_2$ exposures, compressed air was administered through the masks in order to effect desorption of $^{35}SO_2$ from the mucosal surfaces. Dog 5, used as the typical example, had been exposed to 1 ppm $^{35}SO_2$ at 3.5 liters per minute for four trials for a mean duration of 4.9 minutes and a mean mouth absorption of 1.7 percent. The percentage absorption at the end of the fourth exposure was about 3.1 percent. At this point, the mouth was flushed with compressed air at a flow rate of 3.5 liters per minute for 4 minutes. Measurements were performed in a manner identical to that used for $^{35}SO_2$ uptake percentages.

Data for uptake by the nose were presented for various $^{35}SO_2$ concentrations, flow rates, and exposure times. The nose absorbed virtually all of the $^{35}SO_2$ presented at concentrations from 1 to 50 ppm administered at 3.5 liters per minute for 5 minutes for six dogs. Data for nasopharyngeal penetration of $^{35}SO_2$ (that is, $^{35}SO_2$ not absorbed by the nose) is shown for concentrations of 1 and 10 ppm and 3.5 and 35 liters per minute in Table A-10. Dogs exposed to 1 ppm $^{35}SO_2$ at a flow rate of 35 liters per minute for a mean duration of 2.9 minutes had a mean $^{35}SO_2$ penetration of 3.2 percent and therefore an average nasal absorption of 96.8 percent (the highest rate of penetration measured in this experiment for $^{35}SO_2$ administered by nose).

As seen in Table A-11, relative penetration (i.e., nonabsorption) of $^{35}SO_2$ when administered by the mouth, unlike the nose, was greater at 10 ppm than at 1 ppm. Four dogs exposed to 1 ppm $^{35}SO_2$ at 3.5 liters per minute with an average exposure time of 4.6 minutes had a mean absorption by the mouth of 99.6 percent. Also, the rate of administration of $^{35}SO_2$ was more important than concentration in determining the percentage of uptake, particularly for the mouth. At 1 ppm, when the $^{35}SO_2$ flow rate rose ten-fold (from 3.5 to 35 liters per minute), the absorptive capacity of the mouth was rapidly exceeded, but nasal absorption was much less sensitive to the increase in flow rate.

In the desorption studies, no correction was made for any ^{35}S adsorbed to the electrometer ion chamber walls during the uptake experiments, thereby introducing a source of error that would tend to increase desorption values (especially during the early stages of flushing). The authors, by adjusting the figures as if no electrometer wall adsorption had taken place and as if the electrometer had adsorbed all of the $^{35}SO_2$ that had entered it, calculated the upper and lower limits of desorption to be 18 percent and 0.7 percent, respectively. When the airstream was diverted through the unexposed noses (bypassing the exposed mouths) desorption of $^{35}SO_2$ became negligible. This indicated that desorption had occurred exclusively in the mouth and not in any other tissue along the respiratory tract that had been exposed to SO_2.

The results of this study suggested to the authors that in humans, the amount of SO_2 reaching the larynx and lower airways will depend on the mode of breathing (e.g., nasal or oral, fast or slow) as well as on fluctuations in SO_2 levels in the environment. The high flow rate and obligatory mouth breathing typical of heavy exercise and heavy labor are likely to result in an increased SO_2 exposure to the lower airways (since nasal absorption is bypassed) that is out of proportion to the associated increase in minute ventilation. It may be important to note, however, that dogs undergoing several trials, as opposed to one trial, seemed to have lower mean mouth absorption percentages, suggesting SO_2 saturation of local tissues.

The authors considered the continuous desorption of $^{35}SO_2$ from the muscosa into the airstream following cessation of $^{35}SO_2$ exposure a significant finding; in some studies, this continued 25 to 30 minutes postexposure.

Analysis:

A small number of experimental animals was used, and results were not statistically analyzed. However, the design of the study appears to be adequate and the results are useful in that they indicate that SO_2 flow rate is an important variable in its rate of absorption.

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TABLE A-10

Nasal Penetration of $^{35}SO_2$ at
Varying Concentrations and Flow Rates

$^{35}SO_2$ Exposure		No. of Exposures	Mean Duration of Exposure (minutes)	Mean Penetration (% of Initial Concentration)
Conc., ppm	Flow Rate, l/minute			
1	3.5	12	5	0.10
1	35.0	7	2.9	3.20
10	3.5	8	5	0.01
10	35.0	2	3.5	0.01

Note: Table data from Frank et al. Arch Environ Health 18:315-322, 1969.

TABLE A-11

Oral Penetration of $^{35}SO_2$ at
Varying Concentrations and Flow Rates

$^{35}SO_2$ Exposure		No. of Exposures	Mean Duration of Exposure (minutes)	Mean Penetration (% of Initial Concentration)
Conc., ppm	Flow Rate, l/minute			
1	3.5	8	4.6	0.44
1	35.0	4	1.6	66.0
10	3.5	6	3.2	3.7

Note: Table data from Frank et al. Arch Environ Health 18:315-322, 1969.

TABLE A-12

The Rate of Absorption of $^{35}SO_2$ (22 ppm) by the Surgically Isolated Upper Airways of Dogs

Animal no.	Absorptive Rate (%) After Various Exposure Times		
	5-10 min	15-20 min	25-30 min
1	99.9	99.9	100
2	--[a]	99	92
4	--	99.9	83
5	99	--	95
6	99	100	100

[a]Dash (--) indicates data not given.

Note: Table adapted from Frank et al. Health Physics 13:31-38, 1967.

TABLE A-13

Relative Uptake of ^{35}S by the Blood and by Upper Airways

	^{35}S Absorbed by Upper Airways			^{35}S Taken up by Blood			
Animal No.	^{35}S Concentration[a]	Time, min	C x T (total), pc	Peak Blood Conc.	Est. Blood Vol, liters	C x V total	$\frac{^{35}S \text{ in Blood}}{^{35}S \text{ Absorbed}}$
1	3.7×10^4	30	3.9×10^9	2.38×10^5	1.50	3.6×10^8	0.09
2	3.4×10^4	38	4.5×10^9	1.66×10^5	1.45	2.4×10^8	0.05
3	2.5×10^4	60	5.2×10^9	3.19×10^5	1.28	4.1×10^8	0.08
5	1.2×10^4	30	1.3×10^9	1.63×10^5	1.43	2.3×10^8	0.18
6	1.1×10^4	30	1.2×10^9	0.82×10^5	1.71	1.4×10^8	0.12

[a] ^{35}S concentration given as picocuries per milliliter.

Note: Data from Frank et al. Health Physics 13:31-38, 1967.

TABLE A-14

Conditions of Exposure to $^{35}SO_2$ or to $^{35}SO_2$-NH_3 by Rats

Experimental Group	n	$^{35}SO_2$ Concentration, µg/C	$^{35}SO_2$ Specific Activity, mCi/mmoles	NH_3 Concentration, µg/C	$^{35}SO_2$ Concentration registered on Monitor, µg/C
I	7	2.57	31.15		
Ia	6	2.57	31.15	1.04	1.350
II	5	2.70	35.12		
IIa	4	2.70	35.20	1.98	1.140
III	9	2.84	39.10		
IIIa	6	2.84	39.10	2.94	0.950
IV	8	2.77	88.80		
IVa	7	2.77	88.80	1.44	0.734

Note: Data from Jonek et al. Acta Biol Med Ger 35:501-515, 1976.

TABLE A-15

^{35}S in Organs of Rats after Exposure to $^{35}SO_2$-NH_3

Experimental Group	Time After End of Exposure to $^{35}SO_2$	^{35}S, µCi/g in tissue						
		Serum	Heart	Liver	Lungs	Kidneys	Testicles	Brain
I	2 hr	105.9	7.5	19.8	16.48	22.2	6.1	4.9
Ia	2 hr	64.34	4.6	8.5	6.68	9.8	3.9	3.4
II	24 hr	63.4	5.2	7.4	8.34	18.1	5.3	4.3
IIa	24 hr	28.35	4.3	4.4	5.15	9.1	3.2	2.5
III	2 days	24.9	1.0	3.6	9.63	5.8	4.1	1.0
IIIa	2 days	11.9	1.0	1.0	4.8	4.2	3.1	1.0
IV	4 days	11.46	1.0	2.5	5.31	5.0	3.5	1.0
IVa	4 days	8.76	1.0	2.7	4.11	4.8	2.7	1.0

Note: Data from Jonek et al. Acta Biol Med Ger 35:501-515, 1976.

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The relative uptake of ^{35}S by absorption by the upper airways was compared to uptake measured in arterial blood (Table A-13). The amount of ^{35}S in the circulation ranged from 5 to 18 percent of the absorbed dose.

The relative concentrations of ^{35}S in urine were determined for three dogs, during and after $^{35}SO_2$ exposure. Urine samples were obtained about every 15 minutes from a catheter placed in the urinary bladder. During $^{35}SO_2$ exposure, urinary ^{35}S concentrations were 10 to 20 times whole arterial blood concentrations. In two dogs, the urinary excretion rate of ^{35}S progressed to a maximum at or immediately after the end of exposure; one dog displayed a higher excretion rate during recovery. Mannitol (20 ml of 15 percent solution), an osmotic diuretic, was then infused 50 minutes postexposure over a 10-minute period. The result was increased urinary volume with little or no effect on ^{35}S excretion. The authors offered no explanation for this phenomenon.

The authors noted that the method of $^{35}SO_2$ administration may have accounted for the decreased absorption rate with time in dogs 2 and 4. Unlike spontaneous breathing, which allows for intermittent desorption of SO_2 (tending to depress local ^{35}S concentration and prolong maximal absorption rates), the continuous unidirectional dry $^{35}SO_2$ administration allowed no opportunity for desorption. Further, the continuous unidirectional flow may have gradually dried mucosal surfaces, decreasing the capacity for $^{35}SO_2$ absorption with time.

The presence of ^{35}S in the majority of bronchial and carinal air samples suggested that a gaseous form of ^{35}S, probably $^{35}SO_2$, had left the lung tissue fluids and entered the lower airways. The most likely source of this ^{35}S was the pulmonary capillary bed. The authors speculated that the persistence of ^{35}S in the lower airways after termination of upper airway exposure reflected either delayed ^{35}S diffusion into venous blood from the nasopharygeal mucosa or continuous release into the lungs of ^{35}S that was already dissolved in the blood. They did not address other possibilities, such as distribution and redistribution from other body tissues, whether as ^{35}S or specific reversible metabolite.

Analysis:

No statistical analysis was performed with the data resulting from this study. However, the design and execution of this study appear to be adequate and the results are accepted as scientifically valid.

• Jonek J, Konecki J, Kosmider S, Kaminski H: The effect of $^{35}SO_2$ binding by ammonia on sulfur incorporation into rat tissues. Acta Biol Med Ger 35: 501-515, 1976

Review:

Jonek attempted to trace ^{35}S metabolism in male Wistar rats, some of which were exposed to $^{35}SO_2$ alone and others to a combination of $^{35}SO_2$ and NH_3.

A total of 52 rats in eight groups of various sizes were exposed to experimental conditions as shown in Table A-14. Four groups (I, II, III, and IV) were exposed to $^{35}SO_2$ at levels ranging from 2.57 to 2.84 ug/C.* The other four groups (Ia, IIa, IIIa, and IVa) were exposed to those same concentrations of $^{35}SO_2$ plus NH_3 at 1.04 to 2.94 g/C. Each rat was exposed for 50 minutes in an individual chamber. Following exposure to either $^{35}SO_2$ or $^{35}SO_2$-NH_3, the rats were placed in Roth metabolism cages, from which urine and feces were collected each day and the radioactivity of ^{35}S content was estimated. Two hours, 24 hours, and 2 and 4 days postexposure, the animals were anesthetized and blood was taken from the heart. After bleeding the animals to death, the liver, lungs, heart, kidneys, testicles, cerebellum, skeletal muscle, and brains were excised, and either homogenized for liquid scintillation counting or prepared into tissue sections for autoradiography to determine tissue localization of ^{35}S.

In rats exposed to $^{35}SO_2$ alone, ^{35}S entered the blood and tissues. Two hours postexposure, the highest radioactivity was found in the serum, then in the liver, kidneys, lungs, heart, testicles, and brain (as shown in Table A-15). At 24 hours postexposure, radioactivity was still high in the serum, kidneys, and lungs, having decreased in the other organs. On the following days, radioactivity in the serum decreased, while the urine maintained a high level. In rats in which $^{35}SO_2$ was administered with NH_3, the amount of ^{35}S found in the serum and other organs was significantly lower than that obtained with $^{35}SO_2$ alone. The greatest differences were found 24 hours postexposure in rats in which NH_3 was used with $^{35}SO_2$, for which urine radioactivity was approximately 20 percent less than that obtained from $^{35}SO_2$-alone exposed rats. The highest radioactivity was measured in the pulmonary tissue, then in the kidneys, central nervous system, and liver. Autoradiography demonstrated that the pulmonary radioactivity was mainly over the tissues lying in the walls of the alveoli, bronchi, bronchioli, and vesicular tracts. It was thought that the cells incorporating the ^{35}S were fibroblasts. Autoradiographs of the kidney revealed radioactivity almost exclusively in the cortex of the kidney and most frequently over the proximal convoluted tubules or straight tubules. Incorporation of ^{35}S into the central nervous system was almost insignificant. Only in the cerebellum and glial cells adjacent to

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* C was not defined; therefore, actual concentrations are not known. If C = liter, then a level of 2.6 g/C is approximately 1 ppm.

Purkinje's ganglion cells were there detectable traces of radioactivity, but with no typical localization. In muscular tissue, autoradiographs revealed no radioactivity.

In animals exposed to the $^{35}SO_2$-NH_3 mixture, the greatest radioactivity was found (as it was in the $^{35}SO_2$-alone exposure groups) in the serum, then in the pulmonary tissue, kidneys, central nervous system, and liver. However, the levels found were always lower than those in the $^{35}SO_2$-exposed rats. The pulmonary tissue autoradiograph revealed ^{35}S activity in the overlying cells of the alveoli, bronchioli, and bronchi. The number of labeled cells was decreased compared to the $^{35}SO_2$-alone exposed groups. In the kidney, similar to the $^{35}SO_2$-alone exposed group, autoradiography showed rare activity only in the cortical part of the kidney, overlying the proximal convoluted tubules and straight tubules. The intensity and localization of ^{35}S incorporated into the liver and central nervous system were as minor for the $^{35}SO_2$-NH_3 groups as for the $^{35}SO_2$-alone groups.

Analysis:

The data reported here indicated that in rats, after only 2 hours of $^{35}SO_2$ exposure, considerable radioactivity was present in the serum, lungs, and kidneys. This suggested that SO_2 passes quickly from the lungs into the blood and tissues with elimination into the urine (in similar amounts for both exposure groups) mainly during the first day. Once absorbed, ^{35}S was detected 4 days after exposure to $^{35}SO_2$ in several organs including the testicles, brain, lungs, and heart muscle (as well as in the urine).

The authors hypothesized that the use of NH_3 with SO_2 seemed to decrease the measurable amount of SO_2 from that which was initially administered (see Table A-14); however, because they did not show levels of $^{35}SO_2$ above as monitored in the chamber, it is not clear whether NH_3 exerts its neutralizing effect on SO_2 before and/or after these gases enter the organism. Neither were total ^{35}S recoveries reported for each animal, although the relative concentrations of ^{35}S in the organs of animals from both exposure groups strongly suggest that the authors' hypothesis is justified. Rats exposed to the $^{35}SO_2$-NH_3 mixture did have lower concentrations of ^{35}S in serum and in various organs; thus, this difference could be the result of $^{35}SO_2$ binding by NH_3, with the reaction products undergoing precipitation in the air and thereby preventing $^{35}SO_2$ from entering the respiratory tracts of the investigated rats. However, this effect does not seem to be related to the concentration of NH_3 (as seen in Table A-14). No conjectures can be made about the effect of NH_3 on SO_2 incorporation in rat tissue, since $^{35}SO_2$ was used at different specific activities for each concentration of NH_3 and measurements of tissue radioactivity were made at four different times after exposure.

3. Biological Effects and Mortality Data

• Lee SD, Danner RM: Biological effects of SO_2 exposures on guinea pigs. Arch Environ Health 12:583-587, 1966

Review:

Lee and Danner reported the effects of repeated short, high-level exposures to SO_2 on guinea pigs.

Adult guinea pigs were exposed in chambers to SO_2 at various specific concentrations ranging from 6.8 to 310 ppm. (Exposure ranges are shown in Table A-16.) The SO_2 atmosphere inhaled by each of 42 guinea pigs was continuously monitored by an automatic SO_2 analyzer calibrated by the method of West and Gaeke and attached to a sampling probe placed within 1 inch of the nose of the animal. Each guinea pig served as its own control, first receiving filtered air for 30 minutes before exposure, after which certain measurements were obtained. Animals were then exposed to SO_2 for 60 minutes, then re-exposed to clean air for a 60-minute recovery period. As an additional control experiment, six animals received only clean air for the entire experimental period. Measurements taken included tidal volumes (V_t), respiratory rates (RR), and minute volumes ($V_{1\ min}$) once every 15 minutes using a capacitance spirometer. Hemoglobin (Hb) and blood inorganic sulfur (S) were also determined pre- and postexposure.

The six control animals receiving clean air throughout the entire exposure (150 minutes) maintained relatively constant tidal volumes, respiratory rates, and minute volumes. Mean values for the control animals were: V_t, 4.7 ml; $V_{1\ min}$, 514 ml; and RR, 109. Thirty-one of 36 guinea pigs exposed to 19 to 310 ppm SO_2 showed a general increase in V_t at 30 minutes and decreases in RR, with wide individual variations. Ten of twelve guinea pigs exposed to 6 to 18 ppm SO_2 for 30 minutes exhibited decreased V_t, with poorly defined changes in RR. During the postexposure period, RR returned to pre-exposure levels usually within 30 to 60 minutes if the animal had been exposed to less than 180 ppm SO_2 for 30 minutes. In the case of higher (300-310 ppm) SO_2 concentrations for 30 minutes, the recovery was not complete for RR in 60 minutes postexposure time. Maximum changes in V_t occurred within the first 15 minutes of SO_2 exposure with the exception of 300-310 ppm SO_2 exposures, in which V_t continued to increase for about 30 minutes. The apparent partial recoveries that occurred before the end of SO_2 exposure were difficult to explain; the authors attributed them to reflex adaptation.

Hemoglobin (Hb) concentrations consistently increased during all SO_2 exposures for 30 minutes (with 1 exception out of 30). This increase appeared to be linear at SO_2 concentrations between 6 and 20 ppm, but the linearity ceased at concentrations above 20 ppm SO_2. Of 11 controls, nine animals exhibited decreased Hb concentrations, while two controls showed increased Hb concentrations of 4 and 11 percent.

TABLE A-16

Effect of SO_2 Exposure on Mean
Blood Inorganic Sulfur Concentration

SO_2 Exposure Range, ppm	Mean SO_2 Exposure Level, ppm	Number of Animals	Mean Blood Inorganic Sulfur Concentration, mg/100 ml		
			Before Exposure	After Exposure	Change (%)
0 (Controls)	0	6	3.5	3.0	- 14
6-18	13.9	13	3.9	3.9	0
19-50	29.3	12	3.2	3.8	+ 19
70-80	74.7	3	3.0	4.3	+ 43
100-150	115.9	7	3.0	4.2	+ 40
150-180	171.6	4	3.4	3.8	+ 12
180-300	270.0	1	3.5	4.0	+ 14
300-310	305.0	2	2.5	3.9	+ 56

Note: Data from Lee and Danner Arch Environ Health 12:583-587, 1966.

As seen in Table A-16, blood inorganic sulfur concentrations tended to increase after exposures to SO_2 concentrations above 20 ppm; however, this trend was not consistent. In animals exposed to 7 to 19 ppm SO_2, the blood concentrations varied widely. It is of note that the six control animals exposed to clean air throughout the entire experimental period showed a general decrease in blood sulfur (range: +3 to -26 percent), indicating to the authors that the observed increases in the blood sulfur levels in exposed animals were real effects of SO_2 exposure and not artifacts.

Analysis:

The authors concluded from these data that exposure to SO_2 for 1 hour at concentrations of about 19 ppm caused a general increase in tidal volume and a decrease in respiration rate, but no definable effects on minute volume. Exposure to SO_2 at 7 to 17 ppm caused a general decrease in tidal volume and an increase in respiratory rate. However, the data presented seem to be too erratic to warrant these generalizations.

Hemoglobin concentrations did increase after exposure to all concentrations of SO_2 tested (7-310 ppm) and blood inorganic sulfur concentrations did tend to increase with exposure to SO_2 at 19 ppm and above. Almost every one of 42 test animals was exposed to a different SO_2 level and data were presented in some cases for each individual animal with no attempt made to analyze statistically the results. In other cases data for small groups at a given SO_2 concentration range was compared freely with data from larger groups at a different range. As a consequence, these results are of limited usefulness.

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• Alarie Y, Ulrich CE, Bussy WM, Krumm A, MacFarland HN: Long-term continuous exposure to sulfur dioxide in cynomolgus monkeys. Arch Environ Health 24:115-128, 1972

Review:

Alarie et al. reported the effects of accidental high-level SO_2 exposure on cynomolgus monkeys (Macaca irus) during the course of a study of continuous low-level, long-term SO_2 exposure (approximately 0.1-5.0 ppm). After 30 weeks of continuous exposure to SO_2 at 4.69 ppm, a group of nine animals was accidentally exposed to SO_2 at estimated concentrations between 200 and 1000 ppm for 1 hour.

Five groups of nine animals each (weight range: 1.6-3.0 kg) were divided into one control group exposed only to filtered air and four experimental groups exposed to SO_2. The monkeys were placed in individual exposure chambers and, for 8 weeks, all groups were exposed to filtered air only. Various physiologic measurements were taken during this period to establish baseline values and normal week-to-week variations. At the end of the 8 weeks, exposure to SO_2 was initiated for the test groups and continued for 78 weeks. Exposure was interrupted twice daily for 10 minutes for feeding and cleaning and to perform physiologic tests. SO_2 analyzers (Melpar) were used for semicontinuous analysis of chamber SO_2 concentration and were calibrated against the method of West and Gaeke. The control group 1 was exposed to filtered air for 78 weeks. Experimental groups 2, 3, 4, and 5 were exposed to SO_2 for 78 weeks at concentrations of 0.14, 0.64, 1.28, and 4.69, respectively. After 30 weeks of exposure to 4.69 ppm SO_2, group 5 was accidentally exposed to SO_2 at concentrations of 200 to 1000 ppm for 1 hour. Following this overexposure, group 5 monkeys were not re-exposed to SO_2 but remained in chambers with filtered air for the remaining 48 weeks.

Statistical analysis included regression analysis, comparison of the regression coefficients by the Student's t-test, calculation of confidence intervals, and a cumulative sum technique (CUSUM) to establish the time of occurrence of significant differences between nonexposed and exposed groups. The significance level for determining differences between groups was $p < 0.05$.

There were no deaths in group 5, either before or after the accidental SO_2 overexposure. However, two deaths occurred in other groups --one in control group 1, caused by gastric distention, and the other in group 3, caused by intestinal infection, diarrhea, and dehydration. Analysis of the mechanical properties of the lungs comparing pre-exposure and exposure values at 78 weeks revealed that groups 2, 4, and 5 (i.e., animals exposed to 0.14, 1.28, and 4.69 ppm SO_2, respectively) showed increases in tidal volume (V_t) comparable to the control group 1, whereas group 3 (0.64 ppm SO_2) showed a slight, but significantly lower rate of increase in V_t compared to controls. The respiratory rate (RR) values for all experimental groups were comparable to those of the control group. Total respiratory system flow resistance during inspiration (Rrs-i) and expiration (Rrs-e) values were comparable to those

of control group 1 for all experimental groups, with a general trend toward decreased values. Following the accidental SO_2 overexposure, however, group 5 showed greater variation in Rrs-i values than the control group. Pulmonary flow resistance values were similar, while no significant differences were found between group 1 and the experimental groups. Dynamic compliance of the lung results was comparable for the control group and all experimental groups, all values remaining within an extremely narrow range. Similarly, work of breathing during inspiration (W-i) per ml V_t and during expiration (W-e) per ml V_t exhibited no significant changes due to SO_2 exposure. CUSUM analysis of data showed that group 5 monkeys exposed to 4.69 ppm SO_2 had significant increases in N (1 percent N_2) values (the number of breaths required between the beginning and end of the test to reach an end-expiratory nitrogen concentration of 1 percent) following the accidental SO_2 overexposure.

Diffusing capacities for the lung for carbon monoxide were comparable for all groups, with no significant trends observed during SO_2 exposure. Analysis of arterial blood samples showed a significant decrease in $P_{A_{O_2}}$ values for group 5 (accidentally overexposed to SO_2). Group 4 had a small decrease in $P_{A_{O_2}}$ at the start of exposure, but values were comparable to those of the control group 1 for the remainder of the study. No significant $P_{A_{O_2}}$ changes occurred in groups 2 and 3. No changes were observed in $P_{A_{O_2}}$ values for any experimental group, and arterial pH values also remained constant with the exception of a slightly low pH of 7.32 for group 5 animals observed at week 77.

Hematologic and clinical biochemical determinations showed that values for hematocrit, hemoglobin, and red blood cell counts were normal in all SO_2-exposed groups. High mean values of leukocytes (range 11,000-15,000*) were observed in all groups during pre-exposure, decreased during exposure, and attained a range of 8700 to 9700 at week 77. Group 5 reached a high value of 13,900 at week 34 (3 weeks post-SO_2 accidental overexposure). Values for blood lymphocytes, neutrophils, blood urea nitrogen (BUN), total bilirubin, serum total protein, and serum albumin were comparable among all groups during exposure. In addition, serum electrolytes (Na^+, K^+, Cl^-, and Ca^{++}) and SGOT (serum glutamic oxaloacetic transaminase) levels were comparable; SGPT (serum glutamic pyruvic transaminase) values never exceeded control values, but in all experimental groups low values were observed at various time intervals (not reported by the authors). Serum LDH (lactic acid dehydrogenase) and alkaline phosphatase levels were comparable for experimental groups at the begining of exposure, with a definite trend toward lower values as exposure times increased. Analysis of variance of terminal body weight, organ weight, and organ-body weight ratios (obtained separately for male and female monkeys) revealed no significant differences between control and exposed groups.

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* Units are unspecified but presumably are cells per microliter of blood.

On microscopic examination, the lungs, tracheas, hearts, and livers of group 2 and 3 animals, exposed for 78 weeks to 0.14 and 0.64 ppm SO_2, respectively, were comparable to those of the control group. Hearts, livers, and lungs of group 4 monkeys, exposed to 1.28 ppm SO_2 for 78 weeks, were similar to those of controls. Two group 4 animals had minimal submucosal lymphoid hyperplasia of the trachea; five animals had crystalline pigment in the peribronchial lymph nodes and two animals were affected by minimal local interstitial nephritis. All group 5 monkeys, exposed to 4.69 ppm SO_2 for 30 weeks and to 200 to 1000 ppm SO_2 for 1 hour during the accidental overexposure, demonstrated a variety of histopathologic changes. All nine animals showed microscopic alterations in respiratory bronchioles, alveolar ducts, and alveolar sacs. Focally distributed throughout all lung lobes, several groups of alveoli (generally those opening directly off the respiratory bronchioles) contained proteinaceous material and numerous alveolar macrophages. Alveolar walls were thickened and infiltrated with histiocytes, with multinucleated giant cells present in some alveolar lumina. The walls of respiratory bronchioles were moderately infiltrated with histiocytes and leukocytes; bronchiolar epithelium displayed moderate hyperplasia; and the lumina of respiratory bronchioles were often plugged with proteinaceous material, macrophages, and leukocytes. Eight of nine animals showed bronchiectasis, and bronchioles were moderately thickened, but there was little evidence of fibrosis. The alveolitis and bronchiolitis, with pneumocyte hyperplasia, however, appeared subacutely active. Microscopic alterations were also observed in the livers of all group 5 animals, consisting of moderate to severe, diffuse and focal hepatocyte vacuolation. Peribronchial lymph nodes contained crystalline pigment; the kidneys of two animals had slight interstitial nephritis; the hearts and tracheas were considered normal.

The authors interpreted the results of this study as indicating that no detrimental changes in monkeys could be attributed to continuous exposures to SO_2 at 0.14, 0.64, or 1.28 ppm for 78 weeks, or to 4.69 ppm for 30 weeks. However, the SO_2 accidental overexposure of group 5 animals resulted in deteriorations in pulmonary ventilation and in pulmonary and liver histology (as observed by microscopic tissue examination).

Analysis:

The design of this study was appropriate to meet the authors' objectives and the procedures were adequately executed. This study is of value because of the data it contains on low levels of SO_2 exposure, but especially because it reports a concentration range and exposure period for the accidental high-level exposure. Data for these conditions are not generally available in human accidental exposure case reports.

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• Bitron MD, Aharonson EF: Delayed mortality of mice following inhalation of acute soses of CH_2O, SO_2, Cl_2 and Br_2. Am Ind Hyg Assoc J 38(2):129-138, 1978

Review:

Bitron and Aharonson measured the mortality rates and times to death of mice following (but not during) a single exposure to one of four substances including SO_2, formaldehyde, chlorine, and bromine.

The number of delayed deaths was recorded daily for a period of several weeks following exposure, with deaths during exposure excluded from the count. SO_2 was administered at 1900, 1400, and 900 ppm for 10 to 75, 15 to 180, and 25 to 640 minutes, respectively. Usually 16 mice were used for each exposure group with two groups serving as controls. Each mouse was exposed individually within a tight-fitting cylindrical chamber and SO_2 concentrations were frequently monitored through special gas sampling lines.

The data obtained during the postexposure period were the median time of death (MTD, the time at which one-half of the animals died during postexposure only), and quartiles Q_1 and Q_2 (the minimal numbers of days postexposure, up to which at least one-quarter and three-quarters, respectively, of the observed final cumulated mortality developed).

At each concentration of SO_2, both the percentage of deaths during exposure and the cumulated mortality after exposure increased with increasing exposure time. Lt_{50} (the time at which half the animals died) and LCt_{50} (the Lt_{50} multiplied by concentration in ppm) were estimated for SO_2:

- At 1900 ppm, the Lt_{50} was roughly 10 minutes. Therefore, the LCt_{50} was 19,000 ppm·minutes.
- At 1400 ppm the Lt_{50} was 38 minutes. Therefore, the LCt_{50} was 53,200 ppm·minutes.
- At 900 ppm, the Lt_{50} was about 200 minutes. Therefore, the LCt_{50} was about 180,000 ppm·minutes.

For SO_2-exposed animals, the median times of death (MTD) at one Lt_{50} (rough estimates, with many data sets combined) were the following:

- At 1900 ppm SO_2, the MTD at one Lt_{50} was 10 days. The MTD proved to be independent of SO_2 exposure time.
- At 1400 ppm SO_2, the MTD at one Lt_{50} was 9 days. The MTD again was independent of the SO_2 exposure time.
- At 900 ppm SO_2, the MTD at one Lt_{50} was 7 days. At this lower exposure, the MTD was dependent on SO_2 exposure time.

The results in terms of MTD and Lt_{50} showed chlorine to be the most toxic compound investigated, followed by bromine, then formaldehyde, with sulfur dioxide trailing far behind.

The authors stated that the dose-dependent mortality during SO_2 exposure suggested a mechanism of intoxication that differed from that causing the delayed death. They attributed the dose-dependent deaths to asphyxia following bronchostenosis and laryngospasm. The authors remarked on the scant data available on acute respiratory toxicities for SO_2, as well as its questionable reliability given the early dates of the findings and the methods used.

Analysis:

The method of Lichtfield and Wilcoxon was used, but not explained, to evaluate median lethal exposure time. The design and execution of the study appear to be adequate and the results are accepted as scientifically valid. These data help support a generalization that at high SO_2 concentrations, the levels of the gas are more important lethality factors than the total dose (or Ct relationship).

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C. REVIEWS

- Patty FA: Industrial Hygiene and Toxicology, Vol. 2, 2nd Ed., revised. Interscience, New York, 1963

Review:

Patty summarized the physiologic responses of humans to SO_2. Certain acute effects were reported at various concentrations, including:

• 0.3-1 ppm	detectable by the average individual, probably by taste rather than by odor
• 3 ppm	easily noticeable odor
• 6-12 ppm	immediate irritation of nose and throat
• 20 ppm	least amount irritating to the eyes
• 10,000 ppm (1%)	irritant to moist areas of the skin within a few minutes

In addition, the author stated that SO_2 inhalation affects chiefly the upper respiratory tract and bronchi, but may cause pulmonary edema, edema of the glottis, and respiratory paralysis. However, no concentrations were reported for these latter effects.

Chronic effects on humans exposed for 20 or more years to 30 ppm SO_2, with occasional peaks of up to 100 ppm, included the following:

- a significantly higher than normal incidence of nasopharyngitis
- alterations in the senses of taste and smell
- high urinary acidity
- increased fatigue
- an increased duration of colds without a significant change in their incidence.

Exposure of mice and guinea pigs to various concentrations of SO_2 resulted in the following changes:

- 33 ppm or less — no significant changes
- 65 ppm (9 days exposure) — acute distention of the stomach in 33% of the animals
- 100 ppm (4 days) — acute distention and perforations of the stomach

The median lethal concentrations for mice were reported as the following:

- 130 ppm for 24 hours (3120 ppm·hours)
- 340 ppm for 6 hours (2040 ppm·hours)
- 610 ppm for 1 hour (610 ppm·hours)
- 1350 ppm for 10 minutes (1350 ppm·minute or 225 ppm·hours).

The author further reported that for humans the accepted permissible limit for prolonged exposure (TLV) was 5 ppm, 50 to 100 ppm was considered the permissible concentration for 30 to 60 minutes of exposure, and that 400 to 500 ppm was immediately dangerous to human life.

Analysis:

Most of the data reported here was collected from reports dating from 1932 to 1942. More recent information has contradicted some of these findings (e.g., that at 33 ppm or less SO_2 causes no significant changes in mice or guinea pigs). Thus, all data that have not been verified by more modern techniques and equipment must be viewed with some degree of caution.

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• Zielhuis RL: Tentative emergency exposure limits for sulphur dioxide, sulphuric acid, chlorine and phosgene. Ann Occup Hyg 13: 171-176, 1970

Review:

In this report, Zielhuis suggested emergency exposure limits (EELs) for SO_2 based on data derived from previous studies. Unlike the threshold limit values (TLVs), these limits were not intended to be used as guides for the control of the working environment, but rather to indicate the concentrations that, on a single exposure, could be tolerated by humans without serious or irreversible damage and, as such, could be used as a guide for the establishment of levels for emergency exposure for workers and the general population living in the area of a possible industrial accident.

The author's suggestions of the EEL for SO_2 were based on a variety of epidemiologic and experimental data. Sim and Pattle (1957) reported that a Ct of 310 ppm·minute produced relatively little impairment of ventilation in healthy subjects.

Based on that observation, Zielhuis (as shown in Table A-17) suggested the following acceptable deviations from the TLV, based on 300 ppm·minute, where the deviation factor equals the SO_2 concentration divided by the TLV (5 ppm).

TABLE A-17

Suggested Acceptable Deviations from the TLV for SO_2

SO_2 Concentration, ppm	Duration, minutes	$\frac{EEL}{TLV}$ Deviation Factor
60	5	12
20	15	4
10	30	2
5	60	1

Note: Data from Zielhuis Ann Occup Hyg 13:171-176, 1970.

The author stated that numerous studies involving human subjects (who are sedentary and therefore exhibit resting ventilation values) have indicated that exposure to 1 to 40 ppm SO_2 over periods of 10 to 30 minutes may lead to minor but definite impairment of ventilatory function, which would not be sufficient to be unacceptable in emergency situations. Kehoe et al. (1932), Humperdinck (1941), and Anderson (1950) described exposures of 20 to 30 ppm and even more than 100 ppm without serious consequences (for unspecified exposure periods). More recently, Skalpe (1964) reached similar conclusions concerning exposures of 20 to 36 ppm (for unreported periods of time).

Various authors have reported the effects of human exposure to high SO_2 concentrations (Table A-18). Henderson and Haggard (1943) regarded 50 to 100 ppm for 30 to 60 minutes as the maximum allowable dose for humans; 400 to 500 ppm was considered dangerous for even short periods. Flury and Zernik (1931) considered 150 to 190 ppm for 60 minutes to be lethal; Lehmann (1908) reported that 140 to 240 ppm for 30 minutes induced sneezing and lacrimation. Kisskalt (1904) considered 300 ppm SO_2 to be barely respirable and 1500 ppm SO_2 to be insupportable. Ogata (1884) regarded 500 ppm as irrespirable. The Association of German Engineers (VDI 1961) reported that concentrations of 100 ppm SO_2 or greater may lead to severe irritation of the mucosa, inhibition of respiration, and glottal spasm.

In studies of animal exposures (Table A-19), Lee and Danner (1966) reported that exposure of guinea pigs to SO_2 at less than 300 ppm for 60 minutes resulted in a decreased ventilatory volume and respiratory rate, which stabilized within 15 minutes, whereas concentrations greater than 300 ppm SO_2 for 60 minutes caused a progressively increasing response, with a recovery period of more than 60 minutes. Ogata (1884) and Flury and Zernik (1931) reported that death or severe effects were produced in cats, dogs, and rabbits after SO_2 exposures of 800 to 1000 ppm for 20 to 30 minutes and to 500 to 800 ppm for 60 minutes or more.

The most recent research into the effects of SO_2 has been centered around long-term effects at low levels. Much of the information on exposure to high concentrations was derived from earlier literature, dating back even to the last century. This information is frequently quoted although its reliability is difficult to assess. However, from the information presented, the author suggested the tentative EEL values for SO_2 that are presented in Table A-20.

TABLE A-18

Reported Effects of Human Exposure to SO_2

SO_2 Concentration, ppm	Exposure Time minutes	Ct, ppm·min	Reported Effect	Reference
31	10	310	Slight impairment of ventilation	Sim and Pattle 1957
20-100	a	b	No serious consequences	Kehoe et al 1932, Humperdinck 1941, Anderson 19[illegible]0
2-36	a	b	No serious consequences	Skalpe 196[illegible]
50-100	30-60	1500-6000	Maximum allowable exposure	Henderson a[illegible]d and Haggard 1943
400-500	(Short periods)	b	Dangerous	
150-190	60	9000-11,400	Potentially lethal	Flury and Zernik 19[illegible]
140-240	30	4200-7200	Sneezing, lacrimation	Lehmann 1908
300	a	b	Barely respirable	Kisskalt 19[illegible]4
1500	a	b	Insupportable	
100	a	b	Inhibits respiration and glottal spasm, irritates mucosa	VDI 1961

[a] Exposure time not stated in this study
[b] Ct not calculable without exposure time

Note: Data from Zielhuis Ann Occup Hyg 13:171-176, 1970.

TABLE A-19

Reported Effects of Animal Exposure to SO_2

SO_2 Concentration, ppm	Exposure Time, minutes	Ct, ppm·min	Species	Reported Effects	Reference
300	60	18,000	Guinea pig	Decrease in ventilatory volume and respiration rate	Lee and Danner 1966
300	60	18,000	Guinea pig	Further decrease in ventilatory volume and respiration rate; recovery period-60 min	
800-100	20-30	16,000-30,000	Cat Dog	Death or severe effects	Ogata 1884
500-800	60	30,000-48,000	Rabbit		Flury and Zernik 1931

Note: Data from Zielhuis Ann Occup Hyg 13:171-176, 1970.

TABLE A-20

Tentative EEL Values for Sulfur Dioxide

EEL Values. ppm	Duration of Exposure, min	Ct, ppm·min	$\frac{EEL}{TLV}$ Ratio
150	5	750	30
75	15	1125	15
50	30	1500	10
25	60	1500	5

Note: Data from Zielhuis Ann Occup Hyg 13:171-176, 1970.

In Table A-21, Zielhuis contrasts SO_2 with four other toxic gases. The ratios may be regarded as indicating the risks encountered when the TLV is exceeded, i.e., the higher the ratio, the lower the hazard. In general, the agents that affect mainly the upper airways and eyes have a higher ratio (or lower hazard) than those that result in pulmonary edema (having a lower ratio) and are therefore more of a hazard.

Analysis:

In this report, Zielhuis gives some perspective on the available data dealing with high levels of SO_2 exposure. However, it is important to note that the 310 ppm·minute exposure for which Sim and Pattle (1957) found "little impairment of ventilation" was 30.8 ppm for 10 minutes. To extrapolate this exposure to all possible doses of 310 ppm·minute is conjecture at best; certainly 620 ppm for 30 seconds and 3.1 ppm for 100 minutes produce markedly different results. This use of the total dose (Ct) as a basis for establishing EELs for SO_2 is fraught with potential errors.

Also, the use of data from the study by Kehoe et al. (1932) requires further explanation. These authors studied the effects of chronic SO_2 exposures on factory workers, and there were important physical consequences for the workers who, for the most part, had become acclimated to the gas and therefore did not experience the irritant symptoms that a nonexposed person would undergo upon initial SO_2 exposure. In addition, Kehoe et al. did not perform the various pulmonary function tests that are now routinely done. Therefore, the claim that chronic exposures to 30 ppm SO_2 are without serious consequences does not take into account the potential effects on the pulmonary status of exposed individuals.

However, in spite of these and other possible shortcomings in interpretation of the related literature, this attempt to establish EEL values for SO_2 is both warranted and useful and can, as Zielhuis suggests, be updated as more information becomes available.

TABLE A-21

EEL/TLV Ratios for Toxic Gases

Exposure Time, min	EEL/TLV ratio				
	Nitrogen Dioxide	Chlorine	Phosgene	Hydrogen Fluoride	Sulfur Dioxide
5	14	14	20	30	30
60	4	6	2	8	5

Note: Table adapted from Zielhuis Ann Occup Hyg 13:171-176, 1970.

• Amdur MO: Report on tentative ambient air standards for sulfur dioxide and sulfuric acid. Ann Occup Hyg 3:71-83, 1961

Review:

Amdur prepared a report for the State of California Department of Health for use in establishing ambient air quality standards for the state. She concluded that as standards for peak exposures of 1 hour or less, a concentration of 5 ppm is adverse, because it is above the threshold of detection, it is objectionable to some persons, and it can produce measurable bronchoconstriction in humans and guinea pigs.

A level of 10 ppm is considered serious because it is irritating to unaccustomed healthy individuals, and it produces measurable bronchoconstriction in normal human subjects and guinea pigs and severe discomfort and bronchospasm in sensitive individuals, often within a few minutes.

A concentration of 20 ppm was termed an emergency level because it is very irritating to the eyes, nose, and throat, and is well above the levels that produce bronchial constriction in normal human subjects and guinea pigs.

In addition, Amdur reviewed the SO_2 literature available at the time and her findings are presented in Table A-22.

TABLE A-22

Reported Responses to Sulfur Dioxide

SO_2 Concentration, ppm	Reported Response	Reference
0.6	Amount shown to produce no detectable response in human subjects	Amdur and Drinker 1954
1	Amount producing detectable response in human subjects	Amdur et al. 1953 Cullumbine et al. 1955
3	Threshold of odor detection by human subjects	Holmes et al. 1915 Amdur et al. 1952
5	Recommended threshold limit for industry	Threshold Limit Values for 1958
8-12	Termed objectionable by human subjects	Holmes et al. 1915
55	Causes a 50% increase in pulmonary flow resistance in guinea pigs exposed for 1 hour	Amdur 1957
112 ppm for 113 hr (Ct = 12,656 ppm-hr)	LC_{50} for guinea pig	Weedon et al. 1939

Note: Table data as reported in Amdur Ann Occup Hyg 3:71-83, 1961.

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- Greenwald I: Effects of inhalation of low concentrations of sulfur dioxide upon man and other mammals. Arch Ind Hyg Occup Med 10(6): 455-475, 1954

Review:

Greenwald reviewed the currently available literature on the toxicity of SO_2 exposure. His review addressed four major topics: experimental determinations of tolerance to SO_2; effects of chronic and acute SO_2 exposure upon resistance to disease; maximum allowable concentrations for prolonged SO_2 exposure; and possible synergistic effects of SO_2 in combination with other atmospheric contaminants. Reported effects of SO_2 exposure on humans and animals are presented in Tables 1 and 2.

In a discussion of the maximum allowable concentration (MAC) for prolonged exposure to SO_2, Greenwald cited Haggard (1924) who in turn had used information from the experiments of Holmes, Franklin, and Gould (1915). Holmes et al. had stated that a few breaths of SO_2 at 10 ppm or more would cause throat irritation and cough, and would be considered a nuisance by most people even for a short time. Greenwald therefore concluded that Haggard's MAC of 10 ppm for chronic exposure was too high.

Greenwald summarized his conclusions of the effects of SO_2 on humans and animals as the following:

- The various species differ greatly in susceptibility, with rats the most resistant and guinea pigs the least. Man is much more sensitive than is the guinea pig.
- Continued exposure of animals to high concentrations (100 and 500 ppm) of sulfur dioxide lowered their resistance to infection with a variety of microorganisms and decreased the formation of antibodies. Similar exposures to 50 ppm had no such effect.
- Concentrations of sulfur dioxide at 1 ppm and of sulfuric acid at 0.5 mg/m^3 are not detected by human beings but can alter the respiratory pattern.
- Very few healthy adults find concentrations of sulfur dioxide up to 5 ppm objectionable, whereas 10 ppm is apt to be irritating.
- The present maximum allowable concentration for prolonged exposure of 10 ppm for sulfur dioxide is probably too high.
- There is no good evidence that either chronic exposure to concentrations below 5 ppm of sulfur dioxide or occasional exposure to slightly higher concentrations has any ill effect upon healthy persons.
- The possible injurious action of SO_2 at these (unspecified) or at even lower concentrations upon young children, upon the aged, and upon those afflicted with circulatory or respiratory disturbances requires investigation.

- The possible modifying action of other contaminants, particularly aerosols and dusts, should be investigated more thoroughly.

TABLE A-23

Reported Effects of SO_2 Exposure on Humans

SO_2 Concentration, ppm	Exposure Time, minutes	Ct, ppm·min	Reported Results	Reference
10,000-30,000	a	b	Without ill effect	Hirt 1882
500-544	120	60,000-65,280	Dyspnea, temporary corneal cloudiness, desire to cough	Ogata 1884
6-30[c]	Chronic	d	Healthy appearance	Lehmann 1893
30-40[c]	a	b	Nasal irritation	
6.5-15	10-30	65-450	Nasal irritation	
30-57	15	450-855	Marked symptoms, extremely disagreeable	
1500	a	b	Subjects could not inhale a full breath	Kisskalt 1904
300	b	b	Subjects could not breathe for any length of time	
140	30	4200	Nasal irritation, sneezing	Yamada 1905
210 - 240	30	6300-7200	Nasal and eye irritation, sneezing, lacrimation	
1 - 2	d	d	Threshold of detectability	Holmes et al. 1915
10	d	d	Throat irritation, coughing	

TABLE A-23 (cont'd)

Reported Effects of SO_2 Exposure on Humans

SO_2 Concentration, ppm	Exposure Time, minutes	Ct, ppm·min	Reported Results	Reference
1-2[e]	d	d	Below threshold of detectability	Amdur et al. 1953
5	d	d	Throat irritation, increased pulse and respiratory rate, decreased V_t	
6-8	d	d	Detectable but not objectionable	
25-100	Chronic (1-19 years)	d	No significant difference in weight, blood pressure, or X-ray findings when compared to unexposed controls	Anderson 1950
5-70[c]	Chronic	d	Higher incidence of pharyngitis, tonsillitis, acidic urine, abnormal reflexes, longer duration (but not incidence) of colds. No changes in hemoglobin or rbc count	Kehoe et al 1932
23-150[c]	Chronic	d	Chronic catarrh, chest constriction, bronchitis, and emphysema	Humperdinck 1940

[a] Exposure time not stated in this review.
[b] Ct cannot be calculated due to lack of exposure time.
[c] In chronically exposed workers
[d] Not applicable
[e] Group included chronically exposed workers.

Note: Data as reported in Greenwald, Arch Ind Hyg Occup Med 10(6):455-475, 1954.

TABLE A-24

Reported Effects of SO_2 Exposure on Animals

SO_2 Concentration, ppm	Exposure Time, minutes	Ct, ppm·min	Species	Reported Results	Reference
400	240	96,000	Rabbit, mouse	Reversible dyspnea and corneal clouding	Ogata 1884
730	540	394,000	Guinea pigs, rabbit	Inactivity, reversible dyspnea, corneal cloudiness	Kisskalt 1904
383-445	30	11,490-13,350	Rabbit	No gross ill effects, slight emphasematous changes in lungs, respiratory mucosa remained normal	Yamada 1905
3700	25	92,500	Rabbit	Fatal for 1 of 3 animals	
5100	30	153,000	Rabbit	Nonfatal for 2 of 3 animals, but lungs showed edema and emphysematic changes	
500	Chronic[a]	b	Rabbit, pigeon, guinea pig	Restlessness, sneezing, and lacrimation for 2 weeks; one rabbit died on day 18 and 2 guinea pigs on days 5 and 15	Ronzani 1908
50	Chronic[a]	b	Rabbit, pigeon, guinea pig	No obvious ill effects	
187	c	b	Rabbit	Catarrhal changes in lower respiratory passages	Millstein 1928
187	c	b	Rabbit	Dryness of mucosa, emphysema of the lungs, heart dilatation	Elenerski 1928

TABLE A-24 (cont'd)

Reported Effects of SO_2 Exposure on Animals

SO_2 Concentration, ppm	Exposure Time minutes	Ct, ppm·min	Species	Reported Results	Reference
25	d	b	Mouse,rat, rabbit	No lethality	Vedder and Armstrong 1918
50	e	b	Mouse,rat, rabbit	Lethal to only mice and rabbits	
10	f	b	Mouse,rat, rabbit, guinea pig	No lethality	
50-76	g	b	Rabbit	Lowered resistance to tuberculosis	Kisskalt 1904
500	e	b	Rabbit, guinea pig	Lowered resistance to infection, production of antibodies and agglutinins; 5-50% increase in red blood cells and hemoglobin concentration	Ronzani 1908
100	(15 days)	b	Rabbit, guinea pig	Similar but milder effects as above	
50	(30 days)	b	Rabbit, guinea pig	No effects on above variable	

[a] Exposure for 6-7 hours/day for 1 month
[b] Ct calculations are not relevant for chronic exposures
[c] Exposure for 15 minutes/day for 2-3 months
[d] Exposure for 6 hours/day for 15 days
[e] Exposure for 6 hours/day for about 1 month
[f] Continuous exposure for 90 days
[g] Exposure for 9 hours/day for 8 days

Note: Data as reported in Greenwald Arch Ind Hyg Occup Med 10(6):455-475, 1954.

D. ABSTRACTS

• Dixon M, Callanan D, Widdicombe JG: SO_2 and the pattern of breathing. In Milano Symposium, pp. 94-95, 1977

Review:

Dixon (as reported in an abstract) studied the effects of SO_2 on the pulmonary mechanics, patterns of breathing, and lung reflexes of rabbits. When 27 anesthetized New Zealand white rabbits were exposed to 200 ppm SO_2 for 2 to 5 minutes, coughing was observed immediately upon exposure. A transient bronchoconstriction followed within 1 minute, during which the mean total lung resistance (R_L) for the group increased from 25.1 to 67.5 cm H_2O per liter per second, and eventually returned (in an unspecified time) to a value of 30.3 cm H_2O per liter per second.

After 5 minutes of SO_2 exposure, the breathing pattern changed considerably. The inspiratory time (t_i) increased by 0.5 seconds from a control value of 0.45 seconds; and the expiratory time (t_e) decreased by 0.29 seconds from a control value of 0.91 seconds. The tidal volume (V_t) increased by 4.3 ml from a control value of 22.5 ml. The Breuer-Hering inflation reflex was abolished in six of nine rabbits after 5 minutes of SO_2 exposure and was greatly reduced in the remaining three rabbits. All of these changes were reversed after 30 minutes of air breathing; thus the effects of short, high-level exposure of SO_2 were both immediate in onset and reversible.

The authors also recorded nerve fiber activity of 26 pulmonary stretch receptors and 16 lung epithelial irritant receptors in rabbits that were bilaterally vagotomized, paralyzed with gallamine, and ventilated. After exposure to 200 ppm SO_2 for 1 to 8 minutes, the activity of 23 pulmonary stretch receptors was abolished. Within 32 minutes (mean time: 15.1 minutes) after the termination of SO_2 exposure, all the receptors were again active. The 200 ppm SO_2 exposure for 5 minutes caused transient increases in activity in lung irritant receptors in six animals, decreases in two animals, and negative results in eight animals. Within an unspecified time after termination of the exposure, all receptors were again active and responded to mechanical stimulation. Again the results to short, high-level exposures to SO_2 were both immediate in onset and totally reversible.

The authors concluded that exposure of rabbits to SO_2 at 200 ppm for even a short time (maximum time: 8 minutes of exposure) specifically blocked the Breuer-Hering inflation reflex and the pulmonary stretch receptor discharge, while leaving the lung irritant receptor activity intact.

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• Callanan D, Dixon M, Widdicombe JG: The acute effects of SO_2 on pulmonary mechanics, breathing patterns and pulmonary vagal afferent receptors in the rabbit. Proc Physiol Soc (J Physiol) 244:23P-24P, 1975

Callanan et al. (as reported in an abstract) studied effects of SO_2 on pulmonary mechanics, breathing patterns, and pulmonary vagal afferent receptors in the rabbit. Twenty-seven New Zealand white rabbits, anesthetized with pentobarbitone sodium, were used for the studies. Group 1 (17 rabbits) was exposed to SO_2 at 200 ppm for 2 minutes through a tracheal cannula. Group 2 (10 rabbits) was similarly exposed to the same SO_2 concentration for 5 minutes. Immediately upon SO_2 exposure, coughing was observed, followed by a transient bronchoconstriction during which the mean total lung resistance (R_L) for both groups combined increased from 25.1 to 67.5 cm H_2O per liter per second and returned to a value of 30.3 cm H_2O per liter per second. The bronchoconstriction was immediate, occurring within the first minute of SO_2 exposure.

After the group 2 exposure to 200 ppm SO_2 for 5 minutes, the breathing pattern of the rabbits changed considerably, with the mean inspiratory time (t_i) increased by 0.5 seconds from a control value of 0.45 seconds and the mean expiratory time (t_e) decreased by 0.29 seconds from a control value of 0.91 seconds. Mean tidal volume (V_t) increased by 4.3 ml. The Breuer-Hering inflation reflex was abolished completely in six rabbits and greatly reduced in three other rabbits after the 200 ppm SO_2 exposure for 5 minutes. All of these changes were immediate in onset and were found to be totally reversible; by 30 minutes after exposure, all variables approached their control values.

In addition, the authors recorded nerve fiber activity from 26 pulmonary stretch receptors and 16 lung epithelial irritant receptors. The rabbits were bilaterally vagotomized, paralyzed with gallamine, and ventilated with a constant minute volume. When 200 ppm SO_2 was given, the activity of 23 of the pulmonary stretch receptors was abolished within 1 to 8 minutes (mean: 3.5 minutes) from the start of the exposure. When a receptor became inactive, the SO_2 exposure was terminated for that rabbit, eventually resulting in the return of the activity for all of the pulmonary stretch receptors within 32 minutes (mean time: 15.1 minutes). The activity was recorded for lung epithelial irritant receptors in the 16 rabbits exposed to 200 ppm SO_2 for 5 minutes. Six receptors showed transient increases in activity, two showed decreases, and the remainder showed no clear change in activity. After termination of the 5-minute SO_2 exposure, all of these receptors were still active and responded to mechanical stimulation.

The authors concluded that the exposure of rabbits to 200 ppm SO_2 can specifically block the Breuer-Hering reflex and the pulmonary stretch receptor discharge, while leaving the lung irritant receptor activity intact. Of note was the fact that all changes induced by the short, high-level exposures to SO_2 were immediate in onset and quickly reversible.

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• Ball COT, Heyssel RM, Balchum OJ, Elliot GO, Meneely GR: Survival of rats chronically exposed to sulfur dioxide. Physiologist 3:15, 1960 (abstract).

Ball et al. studied the survival of rats chronically exposed to SO_2. Three hundred and five male rats were randomly assigned to seven groups and placed in chambers and exposed to SO_2 at 0 (control), 1, 2, 4, 8, 16, or 32 ppm SO_2 continuously for 8 to 16 months. The incidence and degree of wheezing, eye opacities, fur loss, and development of scaly tails was directly proportional to SO_2 exposure concentrations. The control group maintained the best weight. Leukocyte counts (WBC), percent neutrophils, and hemoglobin were all increased in the SO_2-exposed groups. After 8 months, all of the control group, 93 percent of the 1 to 16 ppm groups, and 82 percent of the 32 ppm group were still alive. At 12 months of SO_2 exposure, 91 percent of control, 84 percent of the 1 to 16 ppm groups, and 58 percent of the 32 ppm were alive. At 16 months into the SO_2 exposure, 75 percent of controls, 68 percent of the 1 to 16 ppm groups, and 44 percent of the 32 ppm group were still alive. Survival data are shown in Table A-25.

TABLE A-25

Effect of SO_2 Exposure to Survival of Rats

SO_2 Concentration, ppm	Surviving Rats, %		
	8 Months	12 Months	16 Months
0 (control)	100	91	75
1-16	93	84	68
32	82	58	44

Note: Data from Ball et al. Physiologist 3:15, 1960.

APPENDIX B

SUMMARY OF EXPERIMENTAL DATA FROM SULFUR DIOXIDE EXPOSURE STUDIES

Species	SO_2 Exposure	Effects	Reference
Human	30.8 ppm, 10 min	• No effects on PFR when SO_2 inhaled by mask or in chamber	Sim and Pattle[25]
		• Occasional increases of 20% in PFR with chest irritation and moist rales	
	8.5 ppm, 60 min	• PFR significantly increased in half of subjects within first 20 min with no further rise at end of 1 hr; prolonged expiratory time	
		• No significant changes in heart rate, respiratory rate, tidal volume, maximum breathing capacity, blood pressure	
	9.9 ppm SO_2 for 60 min plus NH_3 at unspecified concentration after first 50 min	• Cessation of irritant and bronchoconstrictive effects	
	6 ppm SO_2 for 60 min plus burning of magnesium ribbon to release *magnesium oxide* after 50 min	• Cessation of irritant and bronchoconstrictive effects.	
Human	1 ppm, 10-30 min	• Only one subject (with high control values) showed increase in PFR; No consistent changes in end-expiratory thoracic gas volume	Frank[15]
	5 ppm, 10-30 min	• A 39% increase in PFR with 10 min exposure; no consistent change in end-expiratory thoracic gas volume	
	13 ppm, 10-30 min	• A 72% increase in PFR with 10 min exposure; increase of 0.3 liters in end-expiratory thoracic gas volume	
		• Symptoms elicited with first few breaths of SO_2 (concentration not specified): coughing, irritation of throat and chest, salivation within first few breaths; symptoms decreased as bronchoconstriction intensified	
	4-15 ppm, 30 min	• Change in PFR occurred with first min of exposure and was maximal at 5-10 min, then diminished but remained above control values	
	1-2, 4-7, and 14-17 ppm for 30 min	• When combined with SO_2, submicrometer NaCl aerosol had no potentiating effect on PFR	

APPENDIX B (Cont.)

Species	SO_2 Exposure	Effects	Reference
	15 and 29 ppm for 25 min	• PFR increased significantly in 9 of 12 mouth-breathing subjects, but in only 3 of 12 nose-breathing subjects; irritant effects more common in mouth breathers	Frank[15] (Cont'd)
	12.4 ppm, 30 min	• 99% SO_2 absorption by naso-pharyngeal mucosa; some desorption by nasal mucosa (about 2 ppm); Author concluded that mouth breathing allows deeper penetration of airways by gas	
Human	1 ppm, 10-30 min	• After 10 min, no change in PFR in 9 of 11 subjects	Frank et al.[19]
	5 ppm, 10-30 min	• After 10 min, PFR rose 39% above control values	
	13 ppm, 10-30 min	• After 10 min, PFR rose 72% above control values	
		• For 5 ppm and 13 ppm, PFR changes began in first min of exposure; increase was *maximal* at 5 min of exposure	
		• Almost half (45%) of peak response attained in first min of exposure	
		• No further PFR increases for 1, 5, and 13 ppm after 10 min when exposure increased to 30 min; one response began to decline after 5 min for next 25 min of exposure	
		• After 15 min recovery period, group average PFR remained elevated above control value; at 5 ppm, difference was significant for 4 subjects; at 13 ppm, difference was not significant from control values for individual subjects	
		• Recovery of PFR levels began within 2.5 min after exposure	
		• End-expiratory esophogeal pressure: no changes	
		• Pulmonary compliance: no changes (except in one subject)	
		• Respiratory rate, heart rate, and tidal volume: no change	

Species	SO_2 Exposure	Effects	Reference
		• MEFR: decreased by 7% in 9 of 10 subjects	Frank et al.[19] (Cont.)
		• Total vital capacity: non-significant changes	
		• At 13 ppm, FRC increased slightly	
		• No correlation between changes in PFR and amount or duration of cigarette smoking	
Human	0.5 ppm, 15 min	• No significant changes in $MEF_{50\%\ VC}$	Snell and Luchsinger[65]
	1 ppm, 15 min	• Significant decrease in $MEF_{50\%\ VC}$ Positive dose-response relationship: decline in flow rates as concentration increases	
	5 ppm, 15 min	• Significant decrease in $MEF_{50\%\ VC}$ Positive dose-response relationship: decline in flow rates as concentration increased	
	Distilled H_2O aerosol (no SO_2) 15 min	• Significant increase in $MEF_{50\%\ VC}$	
	0.5 ppm NaCl µm aerosol; no SO_2 15 min	• Nonsignificant change in $MEF_{50\%\ VC}$	
	0.5 ppm SO_2 + normal saline aerosol, 15 min	• Insignificant decline in $MEF_{50\%\ VC}$	
	1 ppm SO_2, 15 min (with saline aerosol)	• Nonsignificant decline in $MEF_{50\%\ VC}$	
	5 ppm SO_2, 15 min (with saline aerosol)	• Significant decline in $MEF_{50\%\ VC}$ similar to that observed for SO_2 alone; no synergistic response	
	0.5 ppm SO_2(with distilled H_2O aerosol, 0.3 µm, 15 min 1 ppm SO_2 (with distilled H_2O aerosol) 15 min 5 ppm SO_2 (with distilled H_2O aerosol) 15 min	• Identical significant decline as for SO_2 along for all 3 mixtures in $MEF_{50\%\ VC}$; no dose-response relationship apparent; no synergistic response	

Species	SO_2 Exposure	Effects	Reference
		• When experiments were repeated to compare effects of nose breathing versus mouth breathing, average $MEF_{50\%}$ VC was higher compared to controls after exposure by nose than by mouth (at nonsignificant levels)	Snell and Luchsinger[65] (Cont.)
		• Mouth breathing decreased conductance by 20%; nose breathing increased conductance to greater extent than 0.5 ppm SO_2 in distilled water aerosol. Conductance by nasal inhalation was proportional to gas concentration	
		• All trials: conductance changes occurred after approximately 4 minutes of gas inhalation and were only marginally more pronounced at 12 min of exposure	
		• All trials: subjective reactions included throat irritation persisting for some for more than 1 hr post-exposure	
Human	4-6 ppm, 10 min	• Decreased airway conductance/thoracic gas volume ratio by 39% compared to control values	Nadel et al.[48]
		• Coughing (which decreased with time) irritation in pharynx and substernal area	
		• Onset of changes in airway conductance began from 10 sec to 4 min after start of exposure	
		• In 5 subjects: conductance decreased maximally in first min; 2 subjects conductance continued to decrease throughout exposure; 2 subjects: degree of constriction decreased during exposure	
Human	4-6 ppm (with 1.2-1.8 mg SC atropine) for 10 min	• No significant change in conductive/thoracic gas volume ratio. Coughing, irritation in pharynx and substernal area not affected	
Cats	SO_2 level not measured at single inflation of lower airways	• Pulmonary resistance (R_L) increased by mean of 46% above control values; increased after first breath; returned to control values within 1 min	

APPENDIX B (Cont.)

Species	SO_2 Exposure	Effects	Reference
Human	5 ppm, 3 hr	• 2 hr postinhalation: no changes in VC, FEV at 1 sec or closing volume; 10% reduction in MMEF. No marked effect on mucociliary clearance. Subjective effects (acrid dusty taste, cough) lasted for first 3-4 min	Wolff et al.[20]
Human	22 ppm, 25-35 min	• Nose absorbed approximately 98% SO_2 from inspired air; about 5% desorbed at expiration; net uptake was about 85%	Speizer and Frank[49]
Human	40 ppm, 1 hr	• Urticareal wheals formed (delayed in onset and reversible)	Pirila[91]
Human	20-30 ppm, avg (5-70 ppm range), workdays, for a 4 yr avg	• Initial symptoms confined to respiratory tract, consisted of irritation of the upper respiratory tract, coughing, epistaxis, constriction of chest, hemoptysis • Symptoms associated with customary exposure were milder forms of the initial symptoms • Symptoms after exposure to high levels were intensified forms of symptoms from initial exposure • Compared with control groups, exposed subjects had colds of increased duration (13 versus 5.7 weeks) but not increased frequency • The frequency of exposure to high levels of SO_2 was positively and significantly associated with the frequency of on exertion, fatigability, altered sense of taste and smell and sensitivity to other irritants • The incidences of nasopharyngitis, tonsillitis, abnormal deep tendon reflexes, increased urine acidity, and lymphocytosis were positively and significantly associated with SO_2 exposure	Kehoe et al.[24]
Human	10 ppm, 30 min	• Day 1 postexposure, lesions developed on the cubital fossae with slight swelling of the eyelids and itching of the trunk. Symptoms lasted 1 day.	Pirila et al.[92]
	40 ppm for two 10-min intermittent exposures	• Slight wheezing on right upper thorax was the only immediate symptom noted. The following day, a severe skin eruption developed in the cubital fossae and spread over the upper extremities. One day later, eruption peaked; papules coalesced and eyelids swelled. Lesions regressed over next few days	

Species	SO_2 Exposure	Effects	Reference
Mouse	1900 ppm, 10-75 min	• Lt_{50} = 10 min; LCt_{50} = 19,000 ppm · min	Bitron and Aharonson[14]
	1400 ppm, 15-180 min	• Lt_{50} = 38 min; LCt_{50} = 53,200 ppm·min	
	900 ppm, 25-640 min	• Lt_{50} = 200 min; LCt_{50} = 180,000 ppm·min	
Hamster	400 ppm, 5 hr/day 5 day/wk.	• All animals died within 6.3 hrs of exposure	Asmundsson et al.[13]
	40 ppm, 5 hr/day 5 day/wk., 6 weeks	• Some loss of cilia and vacuolization of ciliated tracheal cells	
	100 ppm, 5 hr/day 5 day/wk, 6 weeks	• Degeneration of ciliated tracheal cells occurred	
	200 ppm, 5 hr/day 5 day/wk 6 weeks	• Loss of most tracheal cilia, vacuolization, extrusion of tracheal ciliated cells within 5 hr. Basal cell hyperplasia and some transitional cell metaplasia after 4 days	
	250 increased to 400 ppm, 5 hr/day 5 day/wk., 7 weeks	• Changes similar to those seen with exposure, but more extensive. Also squamous cell metaplasia and cell necrosis	
	40 + 200 ppm, 5 hr/day 5 day/wk., 8 days	• Loss of cilia, vacuolization, cell extrusion, basal cell hyperplasia, and transitional cell metaplasia; observed later at 40 than at 200 ppm	
	40 ppm SO_2 + 0.74 g/m carbon dust for 4 hr	• No significant leukocyte recruitment. Polymorphonuclear leukocytes found within bronchial walls and in lumen 24 hrs after beginning of exposure	
Dogs	7-16 ppm for 20 min	• R_N increased proportional to level of SO_2 administered	Frank and Speizer[60]
	23-34 ppm for 20 min	• R_N proportional to Ct	
	60-61 ppm for 20 min	• R_N proportional to Ct	
		• For entire exposure groups: - R_L rose and fell independently from SO_2 concentration or duration. R_L tended to increase during recovery above control levels - R_L tended to rise and fall about an equal number of times during exposure to and recovery from SO_2	

Species	SO_2 Exposure	Effects	References
		• Pulmonary compliance during expousre decreased 5% • Minute ventilation fell (avg) 0.3 liter/min during exposure, due to reduction in V_t and RR • End-expiratory volume and end-expiratory transpulmonary pressure showed no consistent trends. At 23-61 ppm SO_2 levels, transient shifts in lung volume (20-25% of control values) occurred infrequently. Changes in lung volume and transpulmonary pressure did not correlate significantly with R_L changes	Frank and Speizer[60] (Cont.)
Dogs	22 ppm $^{35}SO_2$, 30-60 min	• About 99% SO_2 uptake occurred during the first 20 min of exposure. Uptake values began to decrease slightly after 25 min • Less than 1% of inspired SO_2 appeared in expired air. No consistent differences were found between levels in bronchi and carina. ^{35}S appeared in blood within 5 min of onset of exposure; levels increased throughout exposure, fell slowly up to 2 hr. postexposure (when measurements were discontinued) • Amount of ^{35}S in circulation was 5-18% of absorbed dose • Urinary conc. of $^{35}SO_2$ was 10-20 times greater than conc. in blood. ^{35}S excretion reached max. at or just after end of exposure	Frank et al.[51]
Guinea pig	6.8-310 ppm, 60 min	• General increase in V_t and decreases in RR (with wide variation) seen at 30 min in 31 of 36 guinea pigs exposed to 19-310 ppm SO_2 • Decreased V_t and poorly defined changes seen in RR in 10 of 12 guinea pigs exposed to 6-18 ppm SO_2 for 30 min • RR returned to pre-exposure levels 30-60 min. postexposure in animals exposed to 180 ppm SO_2 for 30 min; at 300-310 ppm SO_2 recovery did not occur by 60 min. postexposure	Lee and Danner[61]

Species	SO_2 Exposure	Effects	References
		• V_t reached maximum change within 15 min exposure except for 300-310 ppm level, in which V_t continued to increase for 30 min partial recovery occurred during the exposure period	Lee and Danner[61] (Cont.)
		• Hemoglobin (Hb) levels increased consistently (in 29 of 30 animals) during all SO exposures for 30 min (9 of 11 had decreased Hb, 2 showed an increase)	
		• Blood inorganic sulfur concentrations tended to increase after exposures to SO_2 at 20 ppm	
Mouse	40 ppm, 6-9 days	• Both matched-fed and ad libitum fed (amount of food matched to controls) mice lost 21% of their pre-exposure weight; lung compliance increased 37%	Weiss and Weiss[63]
		• Other findings (at nonsignificant levels): 8% increase in tissue compliance and 17% increase of trapped gas; small decreases in alveolar stability, lung weight, lung wet weight/dry weight ratio	
Rat	0.1 ppm, 10 and 23 days	• Higher level of clearance of TiO_2 particles in exposed compared to control groups	Ferin and Leach[85]
	1 ppm, 10-20 days	• Particle clearance was at control on higher levels	
	1 ppm, 25 days	• Exposed rats had depressed levels of particle clearance	
	20 ppm, 11 days	• Slight decrease in clearance.	
Donkey	26-713 ppm, 30 min	• No impairment of bronchial clearance was observed at levels below 300 ppm. At higher concentrations, clearance occurred at a slower rate and in some instances, there was severe impairment of clearance	Spiegelman et al.[79]
Dog (7)	1-30 ppm $^{35}SO_2$ for 5 min	• Nasal uptake of $^{35}SO_2$ at 1 or 10 ppm exceeded 99% at a flow of 3.5 liters/min; uptake averaged 96.8% at flow rate of 35 liters/min	Frank et al.[30]

Species	SO_2 Exposure	Effects	References
		• When administered by mouth, absorption was 99% (at 1 ppm) and 96.3% (at 10 ppm) at a flow rate of 3.5 liters/min but with 1 ppm delivered at 35 liters/min, absorption fell to 34%. Desorption was estimated to be about 0.7-18% by mouth, and negligible by nose	Frank et al.[30] (Cont.)
Rat	2.57-2.84 μg/Ci $^{35}SO_2$ for 50 min	• 2 hr postexposure, highest radioactivity found in serum, kidneys, lungs; decreased in other organs	Jonek et al.[26]
		• 2 days postexposure, radioactivity in serum was decreased, remained high in urine	
	2.57-2.84 μg/Ci $^{35}SO_2$ + 1.04-2.94 μg/Ci NH	• Amount of ^{35}S found was less than when $^{35}SO_2$ was given alone (e.g., 20% less in urine). Highest radioactivity in pulmonary tissue, than in kidneys, central nervous system, and liver	
Monkey (Macaca irus)	0.14 ppm, 78 wk	• No significant change in Pa_{O_2}, Pa_{CO_2}	Alarie et al.[12]
	0.64 ppm, 78 wk	• Significantly slower rate of increase in V_t compared to controls; no significant change in Pa_{O_2}, Pa_{CO_2}	
	1.28 ppm, 78 wk	• Small initial decrease in Pa_{O_2} initially, but values comparable to control group for remainder of experiment	
	4.69 ppm for 30 wk then estim. 200-1000 ppm for 1 hr	• Significant decrease in Pa_{O_2}, N(1% N_2)	
		• Compared with controls, SO_2 exposed groups showed no significant differences in: - body weight growth curves - RR values and fluctuations - Rrs-e and Rrs-i - pulmonary flow resistance - dynamic compliance of the lung - W-i and W-e	

Species	SO_2 Exposure	Effects	References
		- distribution of ventilation (except for 4.69-ppm group which had significant increase 3 weeks after high SO_2 exposure) V_t increases (except for 0.64 ppm group) ● For all groups: - No changes were observed in Pa_{CO_2} values; arterial pH remained constant (except for slightly low level of pH 7.32 at week 77 in 4.69-ppm group) ● Hematologic and clinical biochemical determinations gave results that are within normal limits or comparable levels among groups	Alarie et al.[12] (Cont.)

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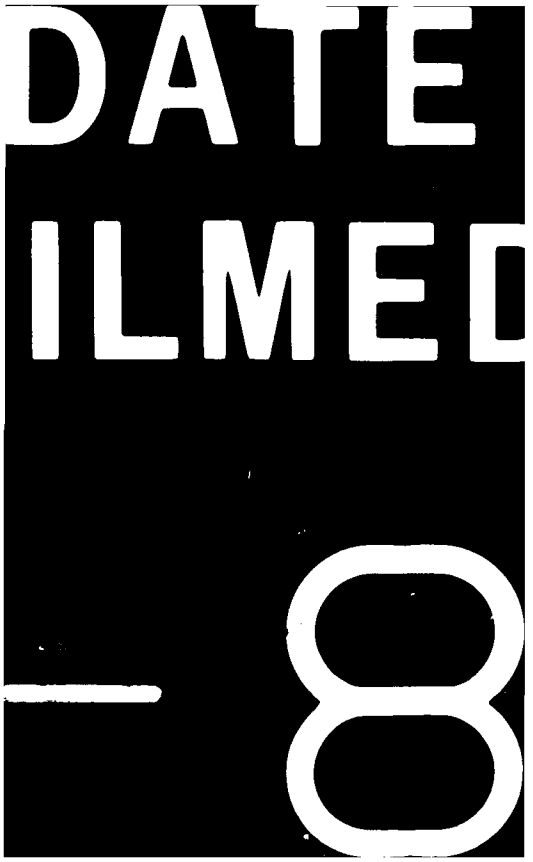
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